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(54) Title: METHODS FOR DIAGNOSING AN IMMUNE-RELATED DISEASE AND CHOOSING AN IMMUNE-RELATED THERAPY

(57) Abstract

A method for diagnosing an immune-related disease and for choosing an immunotherapy by sampling a body fluid which wholly or in part encapsulates the target organs is disclosed. A means for diagnosing the immune-related disease without direct biopsy of the affected tissue, and a means for choosing appropriate immunotherapy which targets only the pathogenic T cells involved in the disease is provided.

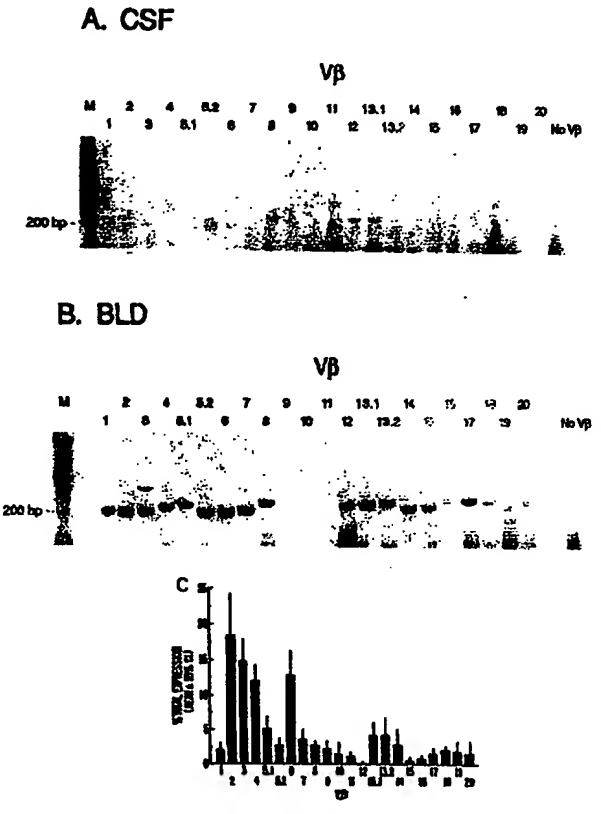

Fig. 4A
CSF AND

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DESCRIPTION

Methods For Diagnosing An Immune-Related Disease And Choosing An Immune-Related Therapy

Field of the Invention

This invention is in the field of immunology and immunotherapy and is directed to novel methods for diagnosing autoimmune diseases by determining the presence of protein differences on the surface of T lymphocytes. The invention also provides novel methods for selecting immunotherapy for autoimmune diseases.

Background of The Invention

The following is a description of relevant art, none of which is admitted to be prior art to the pending claims. This description is not meant to be complete but is provided only to introduce a reader to the relevant fields.

Autoimmune diseases are characterized by an unwanted and unwarranted attack by the immune system on the tissues of the host. While the mechanism for progress of these diseases is not well understood at least some of the details with respect to antigen presentation in this (and other) contexts are being elucidated. It is now thought that antigens, including autoantigens, are processed by antigen-presenting cells (APC), and the resulting fragments are then associated with one of the cell surface proteins encoded by the major histocompatibility complex (MHC). As a result, recognition of a peptide antigen is said to be MHC "restricted". When the MHC/ antigen fragment complex binds to a complementary T cell receptor (TCR) on the surface of a T lymphocyte, it leads to activation and proliferation of the clone or subpopulation of T cells that bear that particular TCR. Once activated, T cells have the capacity to regulate other cells of the

immune system and in some instances to destroy cells or tissues which display the processed antigen.

A review of the role of TCRs in autoimmune diseases by Acha-Orbea et al. *(Ann. Rev. Immunol. 7:371-405 (1989))* discusses the tremendous variation in TCRs available in the immune system of an individual and the generation of this diversity by germ line gene organization and rearrangement of the DNA encoding TCR α and β chains. The α chains are encoded by various combinations of variable (V), junction (J) and constant (C) region gene segments. TCR β chains are additionally encoded by a diversity (D) region gene segment, and, thus comprise a rearranged VDJC sequence. Due to allelic exclusion, a clone of T cells usually expresses only one type of TCR α-β heterodimer.

TCRs associated with a given disease (termed Marker TCRs) are identified using known techniques. A genetic approach using patients known to have myasthenia gravis (MG) or multiple sclerosis (MS) was described by Oksenberg et al., *Proc. Natl. Acad. Sci.* USA 86:988-992 (1989). Sequences of the appropriate TCR β chain have been obtained by genomic analysis using restriction fragment length polymorphisms found in families having a prevalence of the particular autoimmune disease as described by Seboun et al., *Cell* 87:1095-1100 (1989); Burns et al., *J. Exp. Med.* 169:27-39 (1989).

The TCR expressed by a T cell clone responding to a particular autoantigen can be identified using TCR-specific antibodies, either polyclonal, monoclonal or chimeric (see below) which are specific for a TCR variable segment to detect surface expression, employing techniques of fluorescence microscopy, flow cytometry, immunocytochemistry, or other techniques known in the art. Such antibodies have been described for a number of TCR $\alpha\beta$ chain V regions (see, for example, Owhashi et al., *J. Exp. Med.* 168:2153-2164 (1988); Gascoigne et al., *Proc. Nat'l Acad. Sci.* 84:2936 (1987), Rappler et al., 1987, 1988 (*Cell* 49:263); and MacDonald, H.R., *Nature* 332:40 (1988).

Alternatively, the DNA or mRNA of the T cell clone can be probed directly, or after amplification by techniques such as the polymerase chain reaction (Synha et al., *Science* 239:1026 (1988); Saiki et al., *Nature* 324:163 (1986)), by specific hybridization with nucleic acid probes for the various TCR gene families, or using hybridization methods well known in the art. The TCR sequence, or a part thereof, can then be obtained directly from the amplified, rearranged DNA or mRNA. Synha et al. *Science* 239:1026 (1988); Saiki et al. *Nature* 324:163 (1986)).

Expression of a particular TCR can also be identified by determining the nucleic acid sequence encoding at least part of the TCR, for example, after cloning the TCR V gene, or by determining the amino acid sequence of at least part of a TCR protein. It will be apparent that any of the above-mentioned approaches, or additional approaches known to one of skill in the art, will result in the identification of the TCR expressed on a T cell or clone or line of T cells.

Autoimmune diseases involve the action of T helper cells stimulated by the binding of their TCR to an MHC/autoantigen (or non-autoantigen) complex. Prevention and/or treatment strategies have been developed which are based on the disruption of interactions between the MHC/antigen complex and the TCR. Wraith, D.C. et al. *Cell* 57:709-715 (1989), proposed approaches based on this principle, including vaccination with whole T cells, passive blockage using antibodies which bind to the TCR, passive blockage using antibodies that bind to the MHC portion of the complex, and administration of antibodies reactive with the T helper cell marker, CD4. Peptides which mimic the antigen of interest and compete for binding to the MHC or the TCR molecule can also be used (see below). In addition, particular sequences on the TCR itself can be used to stimulate the immune system to downregulate pathogenic T cells.

PCT/US90/04085 and PCT/US92/04492, both herein incorporated by reference indicate that V gene sequences that induce an autoregulatory response can be identified in autoimmune disease processes. Administering these peptide sequences can both suppress and prevent autoimmune disease progression in animal models. An immunogenic peptide can be synthesized which mimics a portion of a disease-associated immunological "marker", such as the TCR of T cells involved in the disease process. Immunization of a subject with such a peptide can direct the host's immune response against the "marker" and thereby prevent or suppress the development and progression of the disease. These publications provide a method for selecting which peptide to use for preventing, suppressing or treating an immune-related disease. The method is based on identifying the amino acid sequence of a marker TCR associated with the disease, predicting which segment of the TCR sequence is immunogenic based on several known algorithms, and determining which site or sites in the TCR structure is an appropriate target for an immune response which will result in protection from the disease.

Thus, in order to effectively diagnose and treat MS patients, for example, it is important to know which limited set of V region genes is preferentially utilized in pathogenic T cells. One or more peptides can then be used to help counteract the disease.

Summary of the Invention

Applicant has determined that disease-relevant T cells can be detected in autoimmune diseases in places distant from the target organ (i.e., the organ directly affected by the disease). It is preferable to sample the tissues targeted in the disease to identify the T cells which cause disease as described by Vandenbark in PCT/US92/04492 and Howell et al. in PCT/US90/01516. However, when the autoimmune disease involves tissues of the central nervous

system this is not desirable since tissue biopsy may produce severe neurological deficit.

Thus, this invention provides a means to detect and treat autoimmune diseases, particularly those affecting the central nervous system. It was determined that inflammatory T cells in the cerebrospinal fluid (CSF) are representative of T cell clones at the disease site in the brain or spinal cord. The T cells in the CSF display a biased use of the V gene preferentially used and the epitope involved in mediating recognition of antigen or epitope involved in the immune-related disease. They may be used to diagnose autoimmune diseases affecting the central nervous system and they may also be used to choose effective autoregulatory V gene sequences which may be administered to the individual to effectively treat the disease.

Thus, in one aspect the invention features a method to diagnose an immune-related disease or an autoimmune disease by detecting the presence of a marker T cell or a marker T cell receptor V gene bias in a body fluid which encapsulates all or a portion of the target organ. Preferably, the body fluid is cerebrospinal fluid (CSF), the autoimmune disease targets the central nervous system (CNS), and the V gene bias is for a Vβ peptide. More preferably, the V gene bias is for Vβ1, Vβ2, Vβ5, Vβ6 or Vβ18.

The term "immune-related disease" as used herein refers to a disease in which the immune system is involved in the pathogenesis of the disease, or in which cell proliferation occurs. Examples of immune-related diseases contemplated by this invention are rheumatoid arthritis (RA), myasthenia gravis (MG), multiple sclerosis (MS), systemic lupus erythematosus (SLE), autoimmune thyroiditis (Hashimoto's thyroiditis), Graves' disease, inflammatory bowel disease (IBD), autoimmune uveoretinitis, polymyositis, pemphigus vulgaris, pernicious anemia, Addison's disease, Goodpasture's syndrome, ulcerative colitis,

Sjogren's syndrome, dermatomyositis, and scleroderma. Also intended as immune-related diseases as used herein are malignancies wherein a tumor cell carries a tumor marker, such as a tumor antigen, capable of being recognized and responded to by the immune system.

The term "autoimmune disease" as used herein means any disease, disease process or pathological state produced, caused, or caused in part by the reaction of B cells or T cells produced by an individual to antigens, epitopes or cells which occur in that individual. The term is meant to include all the examples included in the term "immune-related disease" (specific examples include multiple sclerosis (MS), rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE)).

The term "detecting" as used herein means determining the presence or amount of a specific peptide, protein or nucleic acid being assayed. The preferred method for detecting is by polymerase chain reaction (PCR) analysis as described by Offner et al. *J. Immunology* 151:506 (1993), Chou et al. *J. Neuroscience Research* 37:169 (1994), and Buenafe et al. "Analysis of Vβ8-CDR3 Sequences Derived from the CNS of Lewis Rats with Experimental Autoimmune Encephalomyelitis" (1994), herein incorporated by reference.

The term "marker T cell receptor" as used herein means a T cell receptor which is characteristic of a specified immune-related disease such as an autoimmune disease or a malignant disease (e.g., cancer). A marker T cell receptor for MS is a TCR which is capable of binding the complex between self MHC and the inducing antigen (e.g., the myelin basic protein (BP) fragment (or the BP fragment alone), when the BP fragment is the major autoantigen characteristic of this disease). In other autoimmune diseases, other TCRs serve as markers, as they are specific for the complex between MHC molecules and the autoantigens involved in these diseases. For example, in myasthenia gravis (MG), the autoantigen is thought to be

the nicotinic acetylcholine receptor (AChR). Therefore, an identifiable TCR which binds the AChR in the context of self MHC (or directly) and is expressed by AChR-reactive T cells which mediate the disease is a "marker TCR" for MG. Those of skill in the art will recognize that determination of a marker TCR and of immunogenic peptides may be accomplished with the methods described in PCT/US90/04085.

A V gene is the nucleic acid in a cell expressing a T-cell receptor encoding the variable region of the T-cell receptor. Such a V gene can be readily sequenced or otherwise analyzed by methods well known in the art. See PCT/US90/04085 and PCT/US92/04492, supra. There are several known V genes for both α and β portions of the TCR, termed Vα1, Vα2, etc. and Vβ1, Vβ2, etc. Subfamilies of such V genes are also known and are termed Vβ6.1, Vβ6.2, etc. (See, e.g. Davis et al. *Nature* 334(4):395 (1988); Fink et al. *Nature* 321:219 (1986); Lai et al. *Proc. Natl. Acad. Sci.* 84:3846 (1987)). Thus, by "Vβ gene" is meant the V gene encoding a portion of the β chain of a T cell receptor. The term "V gene bias" as used herein means the presence of a higher concentration (or greater numbers relative to the number in other tissues or fluids in the body not associated with the target organ) of a particular V-gene in T cells in a tissue, organ or fluid, such a lymph nodes, blood or CSF. Such a bias reflects the clonal expansion of T cells with a particular TCR which are used to combat a disease, or which give rise to an autoimmune disease. Thus, detection of such a bias is indicative of a disease causing T cell and associated TCR.

By "higher concentration" is meant T cell numbers in a body fluid containing a particular V-gene significantly greater than those found in peripheral blood or greater than about two standard deviations above the mean number of T cells per milliliter which contain that particular V-gene in a body fluid in normal donors.

By "greater number" is meant at least about twice the number of T cells per milliliter of sample.

The term "body fluid" as used herein means any fluid existing in the extracellular domain in any compartment of a body. Non-limiting examples include cerebrospinal fluid, (CSF), peritoneal fluid, synovial fluid, aqueous humor, and all other body secretions excluding blood.

The term "encapsulates" as used herein means to surround, enclose, compartmentalize or in any way separate a tissue or organ from the environment or from other tissues or organs. Non-limiting examples of structures which encapsulate include the pleura, pericardium, meninges, and the cerebrospinal fluid.

The term "target organ" as used herein means an organ which displays or exhibits a significant or measurable amount of pathology as a result of an immune-related disease. Alternatively, by "target organ" is meant a tissue or organ which displays or undergoes a significant or measurable decrease or change in function as a consequence of an immune-related disease.

By "significant" or "measurable" is meant any amount which can be detected by clinical observation or in a diagnostic test with a statistical chance of at least $P \leq 0.1$.

By "pathology" is meant any gross or microscopic tissue or cellular change which compromises tissue or organ function.

The term "cerebrospinal fluid" as used herein means the fluid which surrounds and protects the central nervous system and is separated from other tissues and organs by the blood-brain barrier.

In preferred embodiments, the disease diagnosed or treated is a neurological disease, e.g., multiple sclerosis and the body fluid is cerebrospinal fluid. In such preferred embodiments, the marker V gene bias is for a Vβ gene, and more preferably it is for a Vβ1, Vβ2, Vβ5, Vβ6 or Vβ18 gene family.

By "neurological disease" is meant any disease process which affects in whole or in part the central or peripheral nervous system, or any portion thereof.

In a second aspect, the invention features a method for diagnosis of an immune-related disease by detecting the presence of a biased motif common to the V gene sequence of T cell receptors specific for an antigen involved in a disease in a non-target tissue or organ.

The term "biased motif" means a TCR peptide or portion thereof or amino acid sequence or epitope which occurs in a higher concentration or in greater numbers than other TCR peptides, portions thereof, amino acid sequences or epitopes. One such motif is the Asp(96)-Ser(97) as described infra. Others can be determined as described below.

The term "antigen involved in a disease" means any protein, peptide or portion thereof which is in any way involved in or mediates a disease process. Non-limiting examples include myelin basic protein (BP) and nicotinic acetylcholine receptors or portions thereof.

A third aspect of the invention is a method for diagnosis of an immune-related disease that targets the central nervous system by obtaining a sample of cerebrospinal fluid (CSF), analyzing the Vβ gene repertoire of T-cells in the sample, and determining the presence of a Vβ gene bias.

The term "Vβ repertoire" as used herein means the total numbers of Vβ genes and the specific numbers of families of Vβ genes expressed or present.

In a preferred embodiment, proliferation assays are performed on the sample of cerebrospinal fluid using one or more of the following: human basic protein, human basic protein peptides, guinea pig basic protein (GP-BP), guinea pig basic protein peptides (GP-BP peptides), interleukin-2 (IL-2), and interleukin-4 (IL-4). Most preferably, the antigen involved in the immune response or portions of that antigen are added to the sample to activate T cells

responsive to that antigen. For Multiple Sclerosis, that antigen might be an antigen on myelin, proteolipid protein, basic protein, or other antigens that are associated with multiple sclerosis. For other autoimmune diseases, those antigens might be connective tissue antigens, tissue antigens, infectious agents or foreign antigens in residence in the tissue or body fluid. Then, IL-2 plus IL-4 are added to the sample to further expand or increase the numbers of those T cells which respond to that antigen.

By "proliferation assay" is meant any means which causes expansion or increased numbers of T cells or specific groups or clones of T cells in the sample of cerebrospinal fluid.

In another preferred embodiment, the presence of a Vβ gene bias is determined by first amplifying Vβ genes by polymerase chain reaction and then quantifying the Vβ genes. In more preferred embodiments, a Vβ gene bias is determined when at least about 20% of the expanded T cells demonstrate a specific V gene, e.g., a Vβ5.2 or Vβ6.1 marker V gene sequence, or a peptide sequence (e.g., a biased motif) specific for a pathogenic antigen such as myelin basic protein.

A fourth aspect of the invention features a method for selecting one or more therapeutic V gene peptide sequences of a selected TCR marker V gene bias. By detecting the TCR marker V gene bias in the body fluid, it has been determined that it is possible to select a peptide to use for preventing, suppressing or treating an immune-related disease. This can be done by identifying the amino acid sequence of the marker V gene peptide, predicting which segment of the TCR sequence is immunogenic based on several algorithms as are known to those of ordinary skill in the art, and determining which site or sites in the TCR structure is an appropriate target for an immune response which will result in protection from or downregulation of disease. Thus, the disclosures of PCT/US92/04492 and PCT/US90/04085 may be extended to study of nontarget

tissues or organs, to determine therapeutic peptides which can be used to effectively suppress, treat or vaccinate immune-related diseases.

The immune-related diseases for which the invention is particularly useful include autoimmune diseases such as multiple sclerosis. Preferably, the diseases involve target organs which are wholly or partially encapsulated by a body fluid. Most preferably, the diseases involve the central nervous system and the fluid chosen is cerebrospinal fluid.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of Drawings

Figure 1. TCR Vβ profile of A) uncultured CSF cells from relapsing/remitting MS patient SL; M, marker lane; No Vβ, PCR control reactions were run in the absence of Vβ primer B) corresponding uncultured PBMC from SL; C) PBMC of 7 normal individuals (% of total cpm, Mean ± 95% Confidence Interval).

Figure 2. TCR Vβ profile of samples from relapsing/remitting MS patient SL in relapse: A) CSF cells cultured with IL-2/4; B) BP-selected CSF T cell line after 3rd stimulation; C) corresponding BP-selected blood T cell line after 3rd stimulation; D) CSF cells cultured with PHA-supernatant for 7 days; E) CSF cells cultured with anti-CD3 for 7 days;

Figure 3. TCR Vβ profile of samples from chronic progressive MS patient WZ: A) CSF BP-selected T cell line from 1st LP after 3rd, 5th and 6th stimulation; B) blood BP line corresponding to (A) after 5th stimulation; C) CSF BP line from 2nd LP after 4th stimulation; D) blood BP line corresponding to (C) after 7th stimulation; E) CSF BP line from 3rd LP after 2nd stimulation; F) blood BP line corresponding to (E) after 2nd stimulation.

Figure 4. Vβ8-CDR3 sequences from CSF of Lewis rats with EAE. Superscript letters a-h designate subgroups of sequences which were identified in more than one cDNA clone. Sequences in section (A) contain the $Asp_{96}Ser_{97}$ motif, while sequences in section (B) contain Ser_{97} but lack Asp at position 96. Sequences in section (C) contain neither $Asp_{96}Ser_{97}$ nor Ser_{97}. Sequences checked under the EAE column designate Vβ8-CDR3 regions associated with encephalitogenic T cell clones. CDR3 length is measured from position 95 of Vβ to position 106 of Jβ. The number of N or nucleotides was determined by comparing CDR3 sequences to those of known Dβ and Jβ germline sequences. N and P nucleotides are not distinguishable in this case because germline Vβ8 sequences are unknown. Differential use of Vβ8 genes is indicated as follows: Vβ8.2 (no symbol), Vβ8.5 (*), Vβ8.6 (#), Vβ8 with Cys_{92} deletion (y), unidentified Vβ8 (?). Underlined nucleotides indicate variations from reported Lewis Jβ sequences and may be due to polymerase error.

Figure 5. Vβ8-CDR3 sequences from spinal cord of Lewis rats with EAE. See legend to Figure 4.

Figure 6. Vβ8-CDR3 sequences from lymph nodes of Lewis rats with EAE. See legend to Figure 4.

Description of the Preferred Embodiments

In the following description, reference will be made to various methodologies known to those of skill in the art of immunology, cell biology, and molecular biology. Publications and other materials setting forth such known methodologies to which reference is made are incorporated herein by reference in their entireties as though set forth in full. The compositions, methods, and products of this invention are applicable to human use.

The following exemplifies the claimed invention. While specific exemplification is provided for MS using CSF this is not limiting in the invention. Applicant is the first to demonstrate that T cells responsible for an

autoimmune disease can be detected in non-diseased sites and is thus the first to provide a means for diagnosis of the disease in a less invasive manner, and to allow for suitable treatment regimens to be developed. Thus, those in the art will recognize that the following is not limiting in the invention but rather allows others to practice within the breadth of the appended claims.

Multiple Sclerosis

Multiple sclerosis (MS) is an immune-mediated disease characterized by central nervous system (CNS) mononuclear cell infiltration and demyelination. Although the pathogenesis of MS is unknown, both genetic and environmental factors have been implicated in the disease process. Major elements of the genetic predisposition presently identified include an association of disease with particular class II MHC halotypes, in particular HLA-DR21 and HLA-DQw1 (Terasad et al., *Science* 1933:1245-1247 (976); Ho et al., *Immunogenetics* 15:509-517 (1982); Spielman et al, *Epidemiol Rev.* 4:45-65 (1982); Francis et al., *Lancet* 1:211 (1986); Elian et al., *Disease Markers* 5:8999 (1987), as well as with certain polymorphisms within the T cell receptor (TCR) α-chain and β-chain gene complexes (Beall et al., *J. Cell Biochem.* 11D:223 (1987); Hauser et al., *J. Neurol.* 89:275-277 (1989); Seboun et al., *Cell* 57:1095-1100 (1989)). These studies suggest that the disease involves CD4$^+$T cells bearing $\alpha\beta$ TCR. In support of this idea, CD4$^+$T cells represent a major component of mononuclear cells in the brains of patients with active MS.

Myelin basic protein, BP, is the major autoantigen involved in EAE and is the leading candidate as an encephalitogen involved in MS. T lymphocytes that recognize BP have been shown to have potent demyelinating and encephalitogenic activity in animals (Ben-Nun et al., *Eur. J. Immunol.* 11:195-199 (1981); McFarlin et al., *New Eng. J. Med.* 307:1183-1188 (1982); Vandenbark et al., *J. Immunol.* 135:223-228 (1985); Zamvil et al., *Nature* 317:355-358

(1985); Bourdette et al., *Cell Immunol.* 112:351-363 (1988). Thus, cells selected from MS patients on the basis of *in vivo* activation have specificity for BP. The frequencies of BP-reactive T cells are increased in MS patients compared to normal individuals or patients with other neurological diseases. In animals, a limited set of TCR α-chain variable (Vα) and β-chain variable (Vβ) genes are utilized by T cells specific for BP (Acha-Orbea et al., *Cell* 54:263-273 (1988); Urban et al., *Cell* 54:577-593 (1988); Burns et al., *J. Exp. Med.* 169:27-39 (1989); Heber-Katz et al., *Immunol Today* 10:164-169 (1989)).

Current therapeutic efforts are directed toward ameliorating acute episodes, preventing relapses or progression of the disease, and relieving symptoms. The clinical manifestations of MS depend upon which nerve group or region of the brainstem, cerebellum or spinal cord is involved. Spinal cord involvement is the predominating feature in most advanced cases of MS.

In acute episodes of the disease, glucocorticoid treatment has been suggested as having the potential to lessen the severity of symptoms and speed recovery, however, ultimate recovery is not improved by this drug nor is the extent of permanent disability altered. ACTH is the preferred glucocorticoid since the only controlled trials which have demonstrated any efficacy of glucocorticoid therapy in episodes of MS and optic neuritis were performed with this drug. However, use of long term steroids is not desirable because of such adverse side effects as immunosuppression, poor wound repair, and bone loss.

Immunosuppressive agents such as azathioprine and cyclophosphamide have been claimed to reduce the number of relapses in several series, but there is no consensus about the efficacy of these drugs either. Thus, the current recommendations for the treatment of MS center around attempting to avoid exacerbation of symptoms. Patients are advised to avoid excess fatigue and extremes

of temperature and eat a balanced diet. (The above discussion is primarily from Chapter 356 of *Harrison's Principles of Internal Medicine*, 12th ed. Wilson et al., McGraw Hill, Inc. 1991.) Interferon-β (IFN-β) has also been used in the treatment of MS. It has been shown to reduce relapses and reduce MRI lesions in the brain in some recent studies.

TCR peptides have been used recently to both treat and immunize patients with possible therapeutic benefit. Patients have been treated and animal subjects immunized with peptides corresponding to the CDR2 sequences of one or more of the Vβ peptides. Studies have shown that administering the peptide corresponding to the CDR2 region of the T cell receptor Vβ chain successfully and reliably immunizes patients. (Kotzin et al. *Proc. Natl. Acad. Sci.* 88:9161 (1991)).

An accurate and early diagnosis is also difficult in MS patients. Early detection of the disease provides the opportunity to inform patients of their prognosis and develop treatment strategies at an early date before the disease has advanced. Early diagnosis may also improve the subsequent clinical course and delay progression by allowing early intervention with available therapies.

Currently, no effective early diagnostic method for MS is known. Diagnosis is directed toward monitoring clinical symptoms followed by confirmatory evoked potential studies, observing oligoclonal bands of IgG in the CSF, computerized tomography (CT) or magnetic resonance imaging (MRI) which may indicate the presence of more advanced focal lesions. There are no confirmatory tissue biopsy methods available since the diseased tissues are largely the brain and spinal cord. Biopsy of these tissues might produce even greater and more severe neurological deficit.

As described below, sequential evaluation of paired CSF and blood samples from MS patients indicate that BP-reactive (i.e., autoantigen reactive) T cells are present in CSF during the period of clinical activity, and the

pattern of BP recognition in CSF is partially reflected in blood, even after CSF reactivity has dissipated during remission. Thus the invention will allow early diagnosis and early treatment of MS. Previous attempts to isolate the T cell receptors involved in the disease process have focused on T cell lymphocytes present in the lymph nodes which drain the disease site or on T cell lymphocytes in the blood. (Gold et al., *J. Immunol.* 148:1712 (1992); Gold et al., *J. Exp. Med.* 174:1476 (1991)).

The V gene which encodes the β chain of the TCR has been shown very important in mediating immune-related diseases. Repeated studies have shown a high correlation between particular Vβ families and the occurrence of specific diseases. (Chou et al., *J. Neuroscience Research* 37:169-181 (1994)). Specifically, recent studies have shown portions of the Vβ5.2 and Vβ6.1 peptides are commonly linked to multiple sclerosis (MS) (Kotzin et al. *Proc. Natl. Acad. Sci.* 88:9161 (1991)) and therefore are useful to treat multiple sclerosis. Rat studies have shown biased use of Vβ8.2 and Vβ6 in rats by encephalitogenic T cells thus allowing use of anti-Vβ antibodies and synthetic TCR peptides for treating experimental autoimmune encephalomyelitis (EAE) (Offner et al. *J. Immunology* 151:506-517 (1993)). Thus, diagnosis of multiple sclerosis may involve detection of a V gene bias for the marker T cell receptor genes Vβ5, Vβ6 or Vβ8 individually or in combination, and in particular Vβ5.2 and Vβ6.1.

Detection of the biased expression or biased occurrence of a motif common to T cell receptors specific for a pathogenic antigen is also shown below. There is a strong bias for expression of Vβ8.2 in the central nervous system of Lewis rats during the onset of experimental autoimmune encephalomyelitis. The Vβ8-CDR3 sequences found in spinal cord (target or disease organ) and cerebrospinal fluid (nontarget organ) lymphocytes show much sequence similarity. A particular asparagine-serine motif is heavily biased in encephalitogenic T cells

recognizing myelin basic protein (BP). Therefore, particular motifs may be used to identify or diagnose immune-related diseases. Such motifs may be identified as described below, and using various techniques including monoclonal, polyclonal or chimeric antibodies specific for the motif, DNA or mRNA probe analysis, and other methods such as are well known to those of skill in the art (see, e.g., Owhashi, M., et al., supra; Gascoigne, N.R.J., et al., supra; Kappler, J.W., et al., 1987, 1988 (supra); and MacDonald, H.R., supra; Synha et al., *Science* 239:1026 (1988); Saiki et al., *Nature* 324:163 (1986).

Specifically, below it is shown possible to diagnose an autoimmune disease that targets the central nervous system by obtaining a sample of cerebrospinal fluid, analyzing the V gene repertoire, and determining the presence of a marker V gene bias. Previous efforts have focused on isolating pathogenic or disease mediating T cells from peripheral blood samples or the lymph nodes that drain the site of the disease. It has not been shown before that a V gene bias in the CSF reflects the pathogenic or disease mediating T cells.

Because of its proximity to the central nervous system, the cerebrospinal fluid (CSF) represents an important source of T cells that mediate autoimmune diseases such as multiple sclerosis (MS). However, low CSF cell concentration makes isolating these T cells difficult. It was determined that T cells mediating the disease can be expanded in CSF samples to detectable levels which still represent the biased clones which mediate the disease. Results obtained are best if a sample of CSF is obtained as early as possible in a clinical episode of the disease.

Collecting CSF

Cerebrospinal fluid may be collected by standard lumbar puncture techniques for analysis of T cell repertoire. Preferably about 20 ml. of cerebrospinal fluid is

obtained. Cells may be obtained from the cerebrospinal fluid, blood and lymph nodes for analysis as explained by Vandenbark et al. *J. Neuroimmunol. 39:251.* The cells may be phenotyped as is known in the art. One method for phenotyping the cells is by using the monoclonal antibodies Leu-3a (anti-CD4+, T helper) and Leu-2a (anti-CD8+, T helper), preferably by indirect immunofluorescence. (Vainiene et al. *J. Neuroimmunol.* 33:207 (1991)).

Cell Proliferation

Proliferation assays can be performed on a cerebrospinal fluid sample to increase cell yields. Those of skill in the art will appreciate many methods for inducing T cell proliferation. Examples include introducing concanavalin-A, interleukin-2 (IL-2), interleukin-4 (IL-4), anti-CD3, PHA supernatant or the pathogenic antigen. Preferably, the proliferation assays are performed using IL-2 and IL-4. IL-2 and IL-4 used together have been shown to expand the T cells in CSF samples by more than ten fold in some subjects. These expanded cells have been shown to be specific for the pathogenic antigen believed to be involved in the disease (Chou et al. *J. Neuroscience Research* 37:169-181 (1994)). Other techniques for T cell expansion are described by Zamvil et al., *Nature* 317:355-358 (1985), and *Nature* 324:258-260 (1986).

For example, lymphocytes may be removed and stimulated with the autoantigen or a specific peptide derived therefrom or related thereto, which is capable of stimulation comparable to that of the autoantigen. The autoantigen (or related peptide) is added to the lymphocyte cultures for several days. Cells may be simulated with autoantigen for 5-6 days or for longer periods of time. The time required for stimulation is a function of the proportion of reactive cells in the sample, the activation state of these cells, and the potency of the stimulating preparation, and is readily determinable by one of ordinary skill in the art. After culture under such selective

conditions, about 5 x 10^5 viable cells are isolated and restimulated with about 3 x 10^7 autologous APCs (antigen presenting cells, irradiated to prevent their proliferation, such as with about 2500-4500 rad) and about 20 μg/ml of the autoantigen (or related peptide). About 7 days later, viable cells are collected and cloned by limiting dilution in the presence of about 10^3 - 10^6 antigen presenting cells, for example about 5 x 10^5 antigen-presenting cells, and human IL-2 or crude or pure combinations of lymphocyte growth factors (such as, for example, IL-4). The cells of such a T cell line are expanded and grown in tissue culture flasks for about one to two weeks. Such lines can be multiply restimulated with antigen-presenting cells, autoantigen preparations, and IL-2. Restimulation can typically be carried out once a week. If desired, such T cells can be cloned by any of a number of methods known in the art, such as, for example, limiting dilution or by picking cells from colonies growing in soft agar, generally about 2 days after restimulation.

In addition, lymphocytes from an organ or body fluid may be cultured in the presence of lymphokines such as IL-2. Under these conditions, selection will occur for cells already activated and only such cells will grow. Subsequently, such T cells may be stimulated with APCs and an autoantigen preparation. Using this approach, MBP-specific T cells can be selectively expanded in vitro. Preferably, the cells are cultured in IL-2 and IL-4.

Detecting the Presence of Reactive T Cells

The presence of autoantigen-specific reactive T cells in a cloned, expanded T cell population can be readily determined by testing the ability of the cells to be activated in the presence of the autoantigen. Many assays are available, and well known in the art, to measure early or late events in the T cell activation process. Examples of such methods include, but are not limited to, T cell proliferation (which can be measured as the uptake of

radiolabeled thymidine), the secretion of interferon-γ, interleukin-2, intracellular calcium mobilization, translocation of particular membrane enzymes involved in inositol phosphate metabolism, and changes in expression of cell surface molecules (which can be determined by flow cytometry). One particular method is described by Chou et al. *J. Neuroscience Research* 22:181-187 (1989).

Where no specific autoantigen has been identified, the oligoclonality of T cells in the anatomic region associated with the disease can be used as a basis for enrichment of reactive T cells. For instance, cells uniquely associated with MS are found in the cerebrospinal fluid (CSF); cells uniquely associated with rheumatoid arthritis may be found in the synovial fluid, and disease-associated T cells infiltrate the thyroid tissue in Hashimoto's thyroiditis and in Graves' disease. In these instances, T cells are isolated from the relevant anatomical location, and the cells expanded in culture as described above. (See also, Londei, M. et al., *Science* 228:85-89 (1985); Londei, M. et al. *Acta Endocrinol.* 115(suppl. 281):86-89 (1987); Stamenkovic, I. et al. *Proc. Natl. Acad. Sci.* 85:1179-1183 (1988); Lipoldova, M. et al. *J. Autoimmun.* 2:1-13 (1989); Oksenberg, J.R., et al., supra). The DNA or mRNA of such cells is isolated, cDNA prepared, and the differences in sequences of cDNA encoding the variable TCR loci are established by comparison of afflicted with unafflicted subjects to determine V gene bias. As an alternative to expanding the cells in culture, cellular DNA or, preferably, cDNA made from mRNA, can be obtained directly from T cells isolated from the subject, and the nucleic acid expanded by the PCR reaction, as above.

Identifying T Cell Receptors

The TCR expressed by a T cell clone responding to a particular autoantigen can be identified using TCR-specific antibodies, either polyclonal, monoclonal or chimeric which are specific for a TCR variable segment. Surface

expression may be detected by employing techniques of fluorescence microscopy, flow cytometry, immunocytochemistry, or other techniques known in the art. Such antibodies have been described for a number of TCR α β chain V regions (see, for example, Owhashi, M., et al., supra; Gascoigne, N.R.J., et al., supra; Kappler, J.W., et al., 1987, 1988 (supra); and MacDonald, H.R., supra).

The DNA or mRNA of the T cell clone can be probed directly, or after amplification by the polymerase chain reaction (Synha et al., *Science* 239:1026 (1988); Saiki et al., *Nature* 324:163 (1986)), by specific hybridization with nucleic acid probes for the various TCR gene families, using hybridization methods well known in the art. The TCR sequence, or a part thereof, can then be obtained directly from the amplified, rearranged DNA or mRNA.

Expression of a particular TCR can also be identified by determining the nucleic acid sequence encoding at least part of the TCR, for example, after cloning the TCR V gene, or by determining the amino acid sequence of at least part of a TCR protein. It will be apparent that any of the above-mentioned approaches, or additional approaches known to one of ordinary skill in the art, will result in the identification of the TCR expressed on a T cell or clone or line of T cells. This information is needed for the selection of an amino acid sequence of the peptide or pharmaceutical preparations useful in this invention for treatment of the target disease.

Determining V Gene Bias

A V gene bias may then be determined by quantifying the V genes expressed or present in the manner indicated above. Usually, about one to two Vβ gene families are expressed or present in a sample in biased numbers. Biased numbers usually means significantly greater numbers than in the blood of the same subject or more than two standard deviations from the mean number for a group of normal donors.

Selecting Therapeutic Peptides

Therapeutic V gene peptide sequences may be selected by first identifying the T cell receptor V gene bias and selecting therapeutic agents which downregulate those T cells displaying the biased gene or cause the T cells displaying the biased gene to be downregulated. The therapeutic agents are generally those described by Vandenbark in PCT/US90/04085 and PCT/US92/04492. The peptide usually corresponds to at least part of the second complementarity determining region of one or more biased V gene families, such as second CDR (CDR2). This also includes peptides corresponding to at least part of the TCR γ and TCR δ chains, their V regions, and CDR structures or their homologs in the $\gamma\delta$ heterodimer (see Strominger, J.L., *Cell* 57:895-898(1989); and Clevers, H. et al. *Ann.. Rev. Immunol.* 6:629-662 (1988)).

Regions of relevant TCR sequences are identified for synthesis on the basis of their predicted antigenic or immunogenic properties, that is the capacity of the peptide to induce an immune response, either T cell-mediated, antibody-mediated, or both. Regions of a protein or peptide that are likely to be immunogenic or antigenic for T cells are identified, for example, using the approaches and algorithms described by Margalit, H. et al. (*J. Immunol.* 138:2213-2229 (1987) and Rothbard, J.B. et al. *EMBO J.* 7:93-100 (1988)). The Margalit et al. approach is based on analysis of immunodominant helper T cell antigenic sites leading to development of an algorithm based on an amphipathic helix model, in which antigenic sites are postulated to be helices with one predominantly polar and one predominantly apolar face. The approach of Rothbard et al., recognizes motifs similar to epitopes recognized preferentially by T helper or T cytotoxic cell clones, which can predict accurately areas within protein sequences that are capable of being recognized by MHC class I and II molecules, such

recognition is assumed necessary for T cell immunogenicity and antigenicity.

In one approach for selecting TCR peptides, the regions of the TCR which are of immunoregulatory importance for the purposes of this invention (based on current models of the structure of the TCR and analogy to antibody structure) fall within CDR1, CDR2, CDR3, CDR4, or in TCR hypervariable regions not strictly part of a CDR, such as residues 39-49 of the Vβ segment (see, e.g. Davis, M.M. et al., *Nature* 334:395-402 (1988)).

The use of this approach to select peptide sequences for use in treating EAE in rats has proven successful. For example, a peptide comprising 16 amino acids corresponding to CDR1 of the marker TCR for EAE in Lewis rats, Vβ8 (25-41) was predicted by the above algorithms not to be immunogenic for T cells. In fact, this peptide does not induce T cell immunity and does not protect Lewis rats from EAE since only Vβ8 bias has been found in EAE. A peptide corresponding to the CDR1 of a different TCR β chain which is not associated with EAE, Vβ14 (25-41), was predicted to be immunogenic for T cells, and indeed was found to induce T cell immunity in Lewis rats, but, as expected, did not protect from EAE. Similarly the CDR2 peptide, Vβ14 (39-59), corresponding to a TCR not associated with EAE, was predicted to be immunogenic, and did induce immunity, but, again, did not protect from EAE. According to the invention, the CDR2-related peptide of the relevant TCR, Vβ8 (39-59), was predicted to be both immunogenic and protective in EAE, and indeed, was shown to be so.

Such peptides generally have a size between 7 and 30 amino acids, e.g., 8-15 or 15-30 amino acids.

Example 1: Vβ Gene Bias In Cerebrospinal Fluid And Blood-Derived Cells

T cells in the CSF demonstrate a bias for V genes and gene products associated with MS in patients with active

disease. Previous studies have revealed T cell Vβ gene biases in lymph nodes and the blood in patients with autoimmune diseases. CSF samples were taken from MS patients and the Vβ gene peptides expressed on the T cell receptors were identified. Results showed a biased or increased concentration of the Vβ genes associated with the disease. The T cells which produce the disease therefore may be isolated from the CSF as well as from the brain and spinal cord. The following experiments were performed. They are reported in Chou et al. *J. Neuroscience Res.* 37:169-181 (1994) herein incorporated by reference.

Patients: Twenty-three MS patients, 16 males and 7 females, with clinically definite MS with a mean age of 45 ± 11 years, range 24-66 years were involved. Three of the MS patients had relapsing-remitting disease, 15 had chronic progressive disease, and 5 had relapsing progressive disease. One MS patient (SL) entered the study in relapse and was tested again 4 months later during remission. The mean duration of MS was 14 ± 8 years (range 3-24 years). The patients had an average Ambulation Index (AI) of 4.5 ± 1.7 (range 2-6) and a Kurtzke Disability Score (KDSS) of 5.6 ± 0.9 (range 3-7). Six patients with other neurological disorders (OND) had a mean age of 54 years, range 30-77 years. The diagnoses for the OND patients were: pseudotumor (AUB), dementia (WS), spinal-myelitis (DO), epilepsy (MC), seizures (MR), and subdural hemorrhage (CS).

Isolation and culture of CSF cells and phenotyping: For each of MS, OND patients and normals 20 ml of CSF was obtained by lumbar puncture without blood contamination. CSF cells were centrifuged at 275xg for 10 min, resuspended in 1 ml of culture medium (RPMI 1640 with 2% pooled AB serum, 1% L-glutamine, 1% sodium pyruvate and antibiotics) and counted in duplicate in a hemocytometer. CSF cells were aliquotted at 1-2x10^4 cells/well in a 96-well U-bottomed culture plate (Becton/Dickinson, Lincoln Park,

NJ) in a final volume of 200 μl of culture medium. Culture conditions: 1) IL-2 and IL-4 were added (50 U/ml for each, AMGEN, Thousand Oaks, CA) twice per week; irradiated autologous peripheral blood mononuclear cells (PBMC) ($0.2x10^6$/well) as feeder cells were also added one time into some IL-2/IL-4-cultured wells for comparison; 2) 50 μl of supernatant from PHA-stimulated autologous PBMC (supernatant collected after 4 days of culturing $0.5x10^6$ PBMC/well with 1:1600 PHA) were added into the culture well with 150 μl culture medium twice per week; 3) as modified from the method described (Kappler, et al., *Science* 244:811-813 (1989)), 50 μg/ml of dialyzed anti-CD3 antibody (Leu-4) (Becton/Dickinson, Mountainview CA) was added twice per week plus an initial addition of irradiated (4,500 Rads) autologous PBMC at $0.2x10^6$/well. CSF BP-reactive lines were initially started with $2x10^4$ cells co-cultured with 50 μg/ml BP and $0.2x10^6$ APC (irradiated autologous PBMC) for 5 days followed by the addition of 50 U/ml IL-2 for 4-7 days, then repeated one more time prior to the proliferation assay. Blood BP-reactive lines were isolated as described previously (Chou et al., *J Neurosci Res* 23:207-216 (1989)). Both freshly isolated and cultured CSF cells were phenotyped using Leu-3a (anti-CD4+, T helper) and Leu-2a (anti-CD8+, T cytotoxic/suppressor) monoclonal antibodies (Becton Dickinson) as described previously (Vandenbark et al., *J Neuroimmunol* 8:103-114 (1985)).

Proliferation assay: 5000 freshly isolated or IL-2/IL-4-cultured CSF cells/well were used in the proliferation assay, which was 25% of the cell number used in previously reported assays for T-cell lines derived from either blood or CSF (Offner, et al., *J. Immunology* 151:506-517 (1993)). To the assay plate (96-well U-bottomed culture plate) medium was added (as background), BP, BP cleavage fragments including residues 45-89 or 90-170, or a synthetic peptide corresponding to the sequence 2-28 at 50 μg/ml with $0.2x10^6$ APC in duplicate cultures.

The proliferation response was measured after 72 hr by uptake of ^{3}H-Tdy (0.5 Bq/well), and the mean CPM calculated. The stimulation index was calculated by dividing experimental mean cpm by background mean cpm. A positive response was indicated when the stimulation index was ≥2.0, with no overlap in CPM of replicates in control versus BP stimulated culture wells.

Human BP was extracted and purified from snap frozen brain (Eylar et al., *J Biol Chem* 246:5770-5784 (1971)) and fragments 45-89 and 90-170 were obtained after peptic cleavage and HPLC purification (Chou et al., *J Neurochem* 28:115-119 (1977)).

Polymerase Chain Reaction (PCR) techniques: Total RNA was isolated using a kit (Stratagene, La Jolla, CA) based on the method of Chomczynski and Sacchi (*Anal Biochem* 162: 156-159 (1987)). First strand cDNA was synthesized in a 50 μl reaction using AMV reverse transcriptase and random hexamers or a downstream Cβ-specific primer, H3Cβ3 (CTGCT-CAGGCAGTATCTGGAG). The presence of Vβ-specific PCR products was determined as follows: 0.1-2 μl cDNA was used in a 15 μl reaction including 0.35 μM of specific Vβ primer, 0.35 μM of H3Cβ5 (CTGCTTCTGATGGCTCAAACAC), a Cβ primer internal to that used for cDNA synthesis (2-3% of which was radioactively labeled with τ^{32}P-ATP), 200 μM dNTPs and 0.5 units Taq polymerase in 1x buffer (50 mM KCL, 10 mM Tris HCl pH 9.0, 0.1% Triton X-100, 1 mM $MgCl_2$). Vβ primer sequences are from the set described by Choi et al. (*Proc Natl Acad Sci.* 86:8941-8945 (1989)) except for Vβ12 (ACTGACAAAGGAGAAGTCTCAGAT, Concannon P. et al., *Proc Natl Acad Sci* USA 83:6598-6602 (1986)), Vβ13.1 (CACTGACCAAGGAGAAGTCCCCAAT, Concannon P. et al., *Proc Natl Acad Sci* 83:6598-6602 (1986)) Vβ13.2 (CTCAGTTGGTGAGGGTACAACTGCC, Concannon P. et al., *Proc Natl Acad Sci* 83:6598-6602 (1986), Vβ17 (CTACTCACAGATAGTAAATGACTTTCAG, Kimura N. et al., *Eur J Immunol* 17:375-383 (1987)) and Vβ21 (TGTGGCTTTTTGGTGCAATCCTAT, Robison MA. *J Immunol* 146:4392-

4397 (1991)). A negative control reaction was run with each sample to verify that no PCR bands appeared in the absence of Vβ primer. All Vβ primers were evaluated in control reactions to insure that no PCR bands appeared in the absence of cDNA. Amplification was carried out for 22 to 26 cycles in a thermocycler (Perkin-Elmer, Norfolk, CT): Denaturation was carried out at 94.5°C x 20 sec, annealing at 60°C x 30 sec, extension at 72°C x 60 sec. Ten μl of the PCR reaction was run on a 6% polyacrylamide gel which was then dried for 1 hr and exposed to x-ray film overnight. PCR Vβ products of the appropriate size were either excised from the gel and quantitated by liquid scintillation counting or quantitated using a Molecular Dynamics PhosphoImager (Molecular Dynamics, Sunnyvale, CA) where background was subtracted using an adjacent region below the band in each lane. Comparisons of human Vβ5.2 and rat Vβ8.2 expression in mixed cell cultures produced similar results when analyzed by semi-quantitative PCR (expressed as percent of total CPM) or anti-Vβ antibody staining (expressed as percent positive fluorescent cells)(Kotzin B.L., et al., *Proc Natl Acad Sci.* 88:9161-9165 (1991), Offner H. et al., *J Immunology* 151:506-517 (1993)).

Recovery and expansion of CSF cells: The average number of cells recovered from the CSF of 23 MS patients was 5,206 ± 1,310 per ml, which was >6.8 fold higher than that from 9 normal donors (757 ± 185 per ml)($p < 0.001$, Table 1). The cell count from 6 OND CSF was 900 ± 207 per ml, also significantly lower than that obtained from MS patients ($p < 0.01$). When expanded in IL-2/IL-4, CSF cells from MS and OND donors could be maintained for an average of 6 days and 14 days respectively before the rapid loss of the cells in culture (Table 1). In contrast, CSF cells from the normal donors did not grow in IL-2/IL-4, with the exception of one individual (KW), who had an elevated CSF cellularity and whose CSF cells responded to the 8-28 peptide of the BP molecule. The

addition of irradiated PBMC accessory cells (AC) plus IL-2/IL-4 prolonged cell growth for 1 to 2 months in all samples, except those from normal donors, when compared to samples cultured in IL-2/IL-4 alone (Table 1).

Retention of BP response under various growth conditions: An MS patient (SL) in clinical relapse had the highest CSF cell count (30,300 cells/ml), nearly six fold greater than the average for the MS group. Such high CSF cellularity occurred relatively rarely, and allowed testing of fresh isolates and optimization of conditions for expanding T cells that included those responsive to BP. Thus, SL CSF cells were expanded using a variety of culture conditions and tested for response to BP and BP peptides, and for TCR Vβ gene expression. In general, the relatively low level of stimulation reflected the low cell number available (5,000/well, one-fourth the usual), but in each case the response was significant ($p < 0.05$) and selective for the indicated peptides.

Freshly isolated SL CSF cells responded selectively to BP and to peptide 45-89, and these responses, as well as T cell responses to additional BP peptides, were retained or boosted when "activated" CSF cells were expanded in culture with IL-2/IL-4 for 14 days (Table 2). When SL CSF cells were stimulated twice with BP in the presence of autologous APC, these cells also responded to BP and peptide 45-89, as in the initial cell isolate. However, cells cultured with either 25% supernatant of PHA-stimulated PBMC or anti-CD3 antibody lost reactivity to BP and BP peptides, although the cell numbers increased 13 and 48 fold respectively under these conditions (Table 2). In PBMC obtained from SL at the same time as CSF, relatively weak reactivity to BP peptide 90-170 was present (albeit at low levels) in both freshly isolated and IL-2/IL-4- or PHA-supernatant-expanded cultures. A BP-reactive T-cell line derived from PBMC without prior culture with IL-2/IL-4 or PHA supernatant responded weakly but selectively both to BP and peptide 90-170, but not to

peptide 45-89 that was recognized predominantly in CSF (Table 2).

These data suggest that for at least one patient in relapse, IL-2/IL-4 addition was the optimal non-specific method for expanding activated CSF T cells that included BP specific T cells. The IL-2/IL-4 expanded CSF cells included more specificities than BP selected T cell lines from CSF or blood, or IL-2/IL-4 expanded cells from blood. In contrast, CSF T cells expanded with PHA supernatant or anti-CD3 antibody were largely BP non-reactive.

Vβ gene expression in CSF and blood samples from patient SL: The predominant Vβ genes expressed in each of the samples obtained from patient SL were identified by semi-quantitative PCR amplification of Vβ message. Freshly isolated CSF and blood from patient SL showed a heterogeneous distribution and intensity of PCR products for each of 22 Vβ genes analyzed. SL Vβ gene repertoire in CSF and blood was similar to the pattern of Vβ message expressed in PBMC of 7 normal donors (mean ± 95% confidence interval (C.I.) are shown for each Vβ gene in Fig. 1C), that showed a consistent predominance of Vβ2, Vβ3, Vβ4, and Vβ6.

In contrast, SL CSF T cells expanded in IL-2/IL-4 (that were BP reactive), were significantly biased in the expression of Vβ5.1 and 5.2 (Fig. 2A). These same two Vβ genes became highly focussed and even more prominent when BP reactive T cell lines were selected from CSF or blood. SL CSF T cells expanded in PHA supernatant or anti-CD3 antibody were not reactive to BP and had much more heterogeneous Vβ expression, with different Vβ genes (Vβ6 and to a lesser degree Vβ1 and Vβ9) predominating in the PHA supernatant expanded sample. These data indicate that expansion of SL CSF cells in IL2/IL4 was the best initial culture method for retaining BP responsiveness and TCR Vβ gene bias, and that selection of BP reactive T cell lines focussed this Vβ expression to an even greater degree.

CSF phenotypes, BP reactivity and Vβ gene bias in MS and control donors; Comparison of expansion techniques: CSF cells from MS patients, OND patients and normals were cultured with AC for 14 days in IL-2/IL-4, 25% PHA supernatant, or anti-CD3 prior to evaluating phenotype, responsiveness to BP or BP peptides, and TCR Vβ gene expression. Freshly isolated CSF cells from both MS patients and controls contained similar proportions of CD4+ and CD8+ T cells as in blood. Culturing with IL2/IL4 plus AC had different effects on MS versus normal donor samples; however, in MS CSF, and in MS and NOR blood, there was a reduction of CD4+ cells leading to a predominance of CD8+ cells. In contrast, the CD4+ subpopulation predominated in NOR CSF. Culturing in PHA supernatant also had differing effects on MS versus NOR cells, with CD4+ T cells predominating in MS CSF and blood samples and CD8+ T cells in NOR samples (Table 3). Culturing either MS and NOR CSF cells with anti-CD3 enhanced the CD8+ T cells. In contrast, selection of BP-reactive T cell lines from blood or CSF resulted in the predominance of CD4+ T cells (Table 3).

After growth in IL2/IL4, CSF cells from 11 of 23 MS, 2 of 6 OND, and 1 of 9 normal donors were expanded sufficiently for analysis of BP reactivity and TCR V gene expression. Of the 11 MS samples, 10 were responsive to BP or BP peptides, and had biased expression of a limited number of Vβ genes, including Vβ1, 2, 5.1, 5.2, 14, & 18 (Table 4). The only normal CSF sample that grew in IL-2/IL4 responded to BP and over-expressed Vβ2 & 18. In contrast, 1 MS and 2 OND samples were unresponsive to BP and had biased expression of Vβ6, 13.1 & 14.

Growth of CSF samples in PHA supernatant yielded ample cells for further evaluation from 8 of 12 MS patients, 3 of 4 OND patients, and 4 of 8 normal donors. Two of 7 MS patients tested responded to BP peptides, but largely overexpressed different Vβ genes (Vβ3, 7, 13.2 & 14) than MS CSFs expanded in IL2/IL4 (Table 4). However, 3 OND

samples that responded to BP or BP peptides overexpressed similar Vβ genes, including Vβ1, 3 & 5. In contrast, MS and normal samples that were unresponsive to BP expressed a mostly non-overlapping set of Vβ genes.

Expansion of CSF cells from 4 of 7 MS and 4 of 5 normal donors with anti-CD3 produced only one BP peptide reactive sample (MS) that was biased in Vβ1 & 2, with the remaining samples expressing a variety of Vβ genes (Table 4).

Thus, expansion of CSF cells in IL-2/IL-4 was preferable to the other non-specific expansion techniques for recovery of BP-reactive T cells expressing a limited Vβ repertoire.

Vβ genes used in response to BP: To ascertain which Vβ genes might be associated with T cell recognition of BP, the expression of a given predominant Vβ gene was compared by Chi squared analysis in BP responsive versus BP non-responsive CSF samples. Although no single Vβ gene was consistently overexpressed, the analysis revealed that it was significantly more likely ($p < 0.001$) for the BP reactive samples to contain Vβ1, Vβ2, Vβ5 or Vβ18 as one of the two predominant Vβ genes versus the BP non-reactive samples.

One consequence of expanding CSF cells in vitro is the appearance of a limited number of Vβ gene families, usually only 1-2. This pattern of limited Vβ gene expression was present consistently in CSF samples that could be expanded by any of the various culture conditions, regardless of the CSF cellularity or of the clinical diagnosis of the CSF donor. BP reactive samples show a highly significant likelihood of Vβ1, Vβ2, Vβ5, or Vβ18 bias. Based on the prominent Vβ gene expression, but relatively weak proliferation response to BP, it is likely that many of the T cells expressing the indicated Vβ gene were not responsive to BP, either because they possessed a different specificity, or that they were in a state of functional anergy.

In three reports that sampled blood BP reactive T cells from MS patients, largely different Vβ gene biases were found, including Vβ17 and Vβ12 (Wucherpfennig K.W. et al. *Science* 248:1016-1019 (1990)), Vβ12, Vβ14 & Vβ15 (Ben-Nun A. et al., *Proc Natl Acad Sci.* 88:2466-2470 (1991)), and a study which found Vβ5.2 and Vβ6.1 (Kotzin B.L. et al., *Proc Natl Acad Sci.* 88:9161-9165 (1991)). The potential importance of Vβ5.2 was corroborated independently by a fourth study in which message for Vβ5.2 was found selectively in plaques from the CNS of HLA-Dw2 MS patients. Moreover, a substantial number of Vβ5.2 sequences contained a CDR3 motif characteristic of BP 84-102 reactive T cells identified previously by us and others. A follow-up study on CSF BP-reactive T cell isolates found over-utilization of different V genes, including Vα2, Vβ7 & Vβ18.

Example 2: Vβ8- CDR3 Sequences

These data show a high correlation between the presence of a particular motif and the ability of a T cell receptor to bind to or interact with a particular antigen. The CDR3 region of the receptor peptide interacts with antigen, and by identifying the CDR3 region or a portion thereof which is involved in interacting with a particular disease-associated antigen, the presence of the disease and the antigen may be determined.

Early Vβ8.2 bias correlates with a dramatic increase in the number of T cells found in both the CSF and SC. By the peak of disease, the Vβ8.2 bias diminishes, presumably due in part to an influx of heterogeneous T cells involved in the inflammatory response. The Vβ8.2 bias has not been detected in T cells derived from the periphery. Karin et al. (*J.Immunol.* 150:4116 (1993)) also found a selective migration of T cells to the brain soon after transfer of encephalitogenic T cells followed by a heterogeneous T cell infiltrate when clinical disease was observed. A

return to limited heterogeneity was seen in the post-acute disease phase.

T cell involvement in EAE is driven by recognition of a CNS antigen, Thus, an expansion of specific Vβ sequences in the CNS is expected. CSF, SC and LN samples obtained at onset of EAE were examined for the presence of Vβ8.2$^+$ AspSer$^+$ sequences. Shirwan et al. (*J. Immunol.* 151:5228 (1993)) recently noted T lymphocytes infiltrating cardiac allografts demonstrated Vβ4 expression was biased in T cell lines and in unmanipulated graft-infiltrating lymphocytes, and was present in a number of sequenced cDNA clones.

Animals

Lewis rat females (6 to 8 weeks of age) were purchased from Harlan Sprague Dawley (Indianapolis, IN) and were housed and cared for in the Animal Resource Facility at the Portland Veterans Affairs Medical Center, according to institutional guidelines.

Induction of EAE

Active EAE was induced by spinal cord injection of 25 mg of Gp-BP in CFA containing 400 mg of Mycobacterium tuberculosis strain H37Ra (Difco Laboratories, Detroit, MI). Rats developing EAE were assessed daily for clinical signs using the following scale: 0, no signs; 1, limp tail; 2, hind limb weakness; 3, paraplegia; 4, paraplegia with forelimb weakness, moribund condition.

Isolation of T lymphocytes

Cells from CSF, SC and LN were obtained as previously described (Offner, et al., *J. Immunol.* 151:506 (1993); Vandenbark, et al., *J. Neuroimmunol.* 39:251 (1992); and Bourdette, et al., *J. Neurosci. Res.* 30:308 (1991)). CSF samples were excluded if the RBC/WBC ratio exceeded 3:1.

Semi-quantitative PCR Analysis of Vβ Expression

Relative Vβ expression was analyzed by semi-quantitative PCR as previously described (Offner, et al., *J. Immunol.* 151:506 (1993)). Vβ-specific PCR products were generated with Vβ-specific primers (Gold, et al., *J. Immunol.* 148:1712 (1992)) and a labeled upstream Cβ primer, RCβ-I (Gold, et al., *J. Immunol.* 148:1712 (1992)), using the following amplification profile: 24 to 28 cycles of 94.5°C for 20s, 55°C for 90s, 72°C for 90s. PCR products were separated on a polyacrylamide gel, dried and exposed to x-ray film. PCR bands were excised from the gel and quantitated by liquid scintillation counting.

Sequence Analysis

All samples were processed in parallel from the time of RNA isolation to transformation and plating of bacterial clones. All sequences were derived from RNA.

For cloning and sequencing purposes, cDNA was amplified with RCβ-E and a 5′ consensus Vβ8 primer as described (Gold, et al., *J. Exp. Med.* 174:1476 (1991)). Amplification was carried out for 32 cycles using the following profile: 94.5°C for 30s, 55°C for 1.5 min and 72°C for 2 min. PCR products were purified using GeneClean (Bio 101 Inc., La Jolla, CA), digested with EcoRI and SacII, re-purified with GeneClean and ligated into EcoRI/SacII-digested pBluescript II SK⁺ (Stratagene Inc., La Jolla, CA). The ligation mixture was used to transform the bacterial strain XL1-Blue. Individual ampicillin-resistant colonies were picked and grown for plasmid DNA isolation by standard procedures (Sambrook, J., E. F. Fritsch and T. Maniatis, eds. 1989. Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.). Plasmid DNA was sequenced using the AmpliTaq (Perkin Elmer Cetus) or Sequenase 2.0 (USB, Cleveland, OH) DNA sequencing kits. The residue numbering system of Kabat et al. (Sequences of proteins of immunologic interest, 5th ed. U. S. Department of Health and

Human Services, Public Health Service, NIH Publication No. 91-3242 (1991)) was used.

In order to optimize for detection of Vβ8.2$^+$ T cells, 6 rats were sacrificed at the earliest sign of active disease, 12 days after Gp-BP immunization (EAE scores of 1+, 0, 0, 0, 0, 0). CSF, SC and LN samples were collected from each rat for Vβ and sequence analysis. SC samples from all 6 rats were pooled for analysis, as were the LN samples. CSF samples, however, were pooled from only 3 rats as the remaining CSF samples had RBC:WBC ratios of greater than 3:1, and were excluded.

Vβ profiles of CSF, SC and LN from EAE rats are shown in Figure 4 as well as the Vβ profile of naive rat LN. On the other hand, PCR analysis demonstrated that Vβ expression in LN from EAE rats was very similar to LN from naive rats, with Vβ8.2 expression at 7% in both LN samples.

Figures 4, 5, and 6 present the Vβ8-CDR3 sequence data obtained from cDNA clones derived from EAE CSF, SC and LN, respectively. The sequences presented here in Figures 4-6 were analyzed for Vβ8 gene usage, presence of the $Asp_{96}Ser_{97}$ or Ser_{97} motif, presence of non-germline encoded nucleotides (N sequences) at the V-D and D-J junctions, Jβ usage and CDR3 length. A corresponding summary of the sequence data is presented in Table 6.

Vβ8 gene usage in the CSF and SC samples was highly restricted to use of the Vβ8.2 gene (23/24 and 22/22, respectively). Only 1 cDNA clone (CSF A4) detected among CSF sequences used the Vβ8.5 gene. Sequences derived from LN were much more heterogeneous in Vβ8 usage, with Vβ8.2 being utilized in only 7/16 of the sequences. In addition to Vβ8.5 and Vβ8.6, LN sequences included 1 clone (LN F12) which utilized a Vβ8 gene family member with a single nucleotide deletion at Cys92.

The $Asp_{96}Ser_{97}$ CDR3 motif has been identified in a number of Vβ8.2$^+$, BP72-89-specific T cell lines and clones. A conserved motif in the CDR3 region of these clones

indicates that these residues may be selected for some functional role.

The Vβ8.2-associated AspSer motif was found in a significant number of CSF (11/23) and SC (9/22) sequences, a few of which were present in multiple cDNA clones. Several of these amino acid sequences have also been associated with BP-specific, encephalitogenic T cell clones as indicated in Figures 4-6. Interestingly, one such sequence was found in 4 CSF clones (CSF subgroup a: A2, A3, B15, B16) as well as in 4 SC clones (SC subgroup a: C7, C15, D4, D9). Two other EAE-associated AspSer$^+$ sequences identified among CSF sequences were also found in multiple clones (CSF subgroup b: A14, B9 and CSF subgroup c: A1, B13, B14). Only 1 LN sequence (LN E2) was found to bear the Vβ8.2$^+$ AspSer motif and all LN sequences were unique. The level of sequence diversity observed in LN reflects the fact that direct analysis of lymphoid tissue is unlikely to detect large numbers of antigen-specific T cells without further expansion in vitro.

Since these samples have not undergone any form of expansion or selection in vitro, the data suggest that these Vβ8.2$^+$ T cells bearing AspSer have been activated and undergone some form of selective recruitment to or expansion in the CNS, the target organ of autoimmune attack in EAE.

Analysis of Vβ8.2$^+$ cDNA sequences bearing Ser_{97}, which includes those with the $Asp_{96}Ser_{97}$ motif, showed this particular residue to be present in the majority of sequences derived from CSF (18/23) and SC (18/22) but in only 2 LN sequences. All expanded sequences found in the CSF and SC contained either $Asp_{96}Ser_{97}$, or Ser_{97} alone.

CDR3 length appeared constant among the Vβ8.2$^+$ AspSer$^+$ or Ser$^+$ sequences analyzed here. The majority (35/38) of these sequences were found to have a rather short CDR3β length of 7 amino acids as measured from aa95 of Vβ to aa106 of each Jβ segment (Chothia, et al., *EMBO J.* 7:3745 (1988); and Kabat, et al., Sequences of proteins of

immunologic interest, 5th ed. U. S. Department of Health and Human Services, Public Health Service, NIH Publication No. 91-3242 (1991)).

Other embodiments are within the following claims.

BP Reactivity and Vβ Gene Bias in CSF

TABLE 1. Cellularity and Culturability of CSF Cells From MS, OND, and Normal Donors

	MS	OND	Normal
Cell no./ml (mean ± SE)	5,206 ± 1.310[a] (n = 23)	900 ± 207 (n = 6)	757 ± 185 (n = 9)
Days in culture[b] (mean ± SD)			
+IL-2 and IL-4	5.8 ± 2.0 (n = 23)	14 ± 1.0 (n = 6)	3.0 ± 0.8[c] (n = 8)
+AC[d]+IL-2 and IL-4	> 30	>30	<5

[a]Cellularity significantly greater than in OND and normal donors, $P < 0.01$.
[b]Days until cell number decreased to <10% of starting number.
[c]This value did not include CSF cells fron one donor (KW) from whom cells could be maintained for an exceptionally long period (>30 days).
[d]Accessory cells, 0.1 x 10^6 irradiated autologous PBMC/well.

TABLE II. Growth Rate and BP-Specificity of CSF Cells From an MS Patient in Relapse Using Different Culture Conditions

	Uncultured	+IL-2/11.-4	+PHA-super[a]	+Anti-CD3[b]	+BP+APC[c]
Growth rate					
cell no. (1×10^3)	20	220	260	960	300
(↑ folds)	—	(11)	(13)	(48)	(15)
Specificity					
cpm[d] (index)					
medium	251	78	300	184	652
BP	659 (2.6)	159 (2.0)	125	133	1951 (3.0)
8-28	210	387 (4.9)	120	212	804
45-89	582 (2.3)	3342 (43.0)	103	105	1656 (2.5)
90-170	372	651 (8.3)	266	288	791
V gene bias	none	Vβ5.1, Vβ5.2	Vβ6	none	Vβ5.1
Blood control					
medium	548	169	456	NT[e]	1655
BP	978	322	702	NT	4238 (2.6)
8-28	699	219	526	NT	1321
45-89	609	156	775	NT	1544
90-170	1143 (2.1)	362 (2.1)	1200 (2.6)	NT	5402 (3.2)
V gene bias	NT	NT	NT	NT	Vβ5.2

[a]Twenty-five percent supernatant of PBMC stimulated with PHA-P 1:1,600 for 3 days.
[b]Fifty micrograms per milliliter of dialysed anti-CD3 + irradiated (4,500 rad) PBMC (0.2×10^6) in each well.
[c]Two stimulations done by adding 50 μg/ml BP irradiated PBMC (0.2×10^6/well) for 3 days followed by a 4-day resting culture in IL-2 (50 u/ml).
[d]The value of cpm presented was the mean of duplicate cultures with ≤ 30% variation. The CSF cell number used was 5,000 cells/well and the blood cell number used was 20,000 cells/well in the assay. Underlined values were significantly ($P < 0.05$) elevated vs. medium control value.
[e]NT + not tested.

TABLE III. Phenotypic Distribution of MS CSF Cells and PBMC in Different Culture Conditions

	MS		Normal control and OND		
	CD4	CD8	CD4	CD8	SE
CSF					
Fresh	55	19	50	25	<10%
+IL-2/IL-4	21	28	61	26	<20%
+PHA-sup	91	4	5	90	<15%
+anti-CD3	27	48	24	62	<20%
BP line	56	19	ND	ND	--
PBMC					
Fresh	51	26	45	28	<10%
+IL-2/IL-4	20	54	10	58	<20%
+PHA-sup	47	34	16	78	<10%
BP lines	72	23	67	18	<5%

TABLE IV. BP Specificity and TCR Vβ Expression of Culture-Expanded CSF Cells

Culture addition	Patient group	Patient	BP response[a] BP	BP response[a] Peptide	Vβ gene bias[b] (% of total cpm)
IL-2/IL-4	MS	SL	+	8-28 45-89 90-170	5,1 (43) 5.2 (32)
		BG	+	45-89	5,1 (49) 1 (10)
		JC	+	NT[c]	2 (100)
		RK	+	NT	2 (43) 18 (30)
		CC	+	NT	2 (33) 18 (43)
		AD	+	NT	1 (66)
		KM	+	NT	1 (87)
		WZ	-	90-170	5,1 (21) 1 (17)
		AB	-	90-170	2 (50) 18 (26)
		TR	-	45-89	1 (53) 14 (13)
		GS	-	—	6 (35) 14 (22)
	OND	MAR	-	—	13,1 (97)
		DO	-	—	13,1 (20) 6 (44)
	Normal	KW[d]	-	8-28	2 (33) 18 (13)
PHA Sup	MS	WS	-	8-28	7 (68) 14 (25)
		KJ	-	90-170	3 (26) 13.2 (25)
		SL	-	—	6 (34) 1 (17)
		KM	-	—	8 (72)
		OB	-	—	8 (36) 11 (33)
		ROS	-	—	3 (57) 7 (19)
		DE	-	—	7 (35) 14 (21)
		HK	NT	NT	6 (61) 13,1 (27)
	OND	AUB	+	90-170	3 (41) 5.2 (29)
		MAR	-	45-89	3 (46) 1 (43)
		WOS	-	8-28	1 (51) 5,1 (10)
	Normal	KW	-	—	6 (16) 5,1 and 5,2 (13)
		AB	-	—	1 (41) 7/14 (18)
		JG	-	—	7 (55)
		JQ	-	—	15 (43) 6 (35)
Anti-CD3	MS	JC	-	45-89	2 (33) 1 (15)
		SL	-	—	no bias
		KM	-	—	2 (32) 3 (42)
		TR	-	—	6 (18) 7 (12)
	Normal	JA	-	—	2 (50) 7 (15)
		KMO	-	—	3 (40) 1 (33)
		MCR	-	—	13,1 (21) 9 (19)
		TB	-	—	3 (37) 12 (16)

[a]CSF cells expanded as indicated were tested by proliferation for response to intact BP or BP peptides 8-28, 45-89, and 90-170. Significant ($P < 0.05$) response to BP(+) or peptide is indicated.

[b]Vβ gene expression was evaluated by semiquantitative PCr. The two most predominant Vβ genes, which represent >10% of the total, and which are expressed at a significantly higher level than the mean of PBMC from seven normal donors, are listed.

[c]NT = not tested.

[d]Among nine normal CSF donors, only SW CSF cells could be expanded in IL-2/IL-4.

TABLE V. Comparison of BP-Reactivity and TCR Vβ Expression Between CSF T Cells and Blood MNC-Derived BP-Reactive Lines in Two MS Patients

Patients	CSF		Blood	
	Specificity (SI) Ag	Vβ (% of cpm)	Specificity (SI) Ag	Vβ (% of cpm)
SL (relapsing remitting)				
First LP (in relapse) (CSF 30,303 cells/ml)	IL-2, IL-4: 2 BP 5 8-28 43 45-89 8 90-170	Vβ5.1 (46) Vβ5.2 (32)	2 BP 3 90-170	NT
	Line: 3 BP 3 90-170	Vβ5.1 (88)	3 BP 3 90-170	Vβ5.2 (56)
Second LP (4 mons later) (in remission) (CSF 4,128 cells/ml)	IL-2, IL-4: 1 BP 1 peptide	Vβ21 (22) Vβ6 (16)	3 BP 3 90-170	Vβ5.2 (21) Vβ6 (20)
WZ (chronic progressive)				
First LP (CSF 7,216 cells/ml)	Line: 3 BP 2 90-170	Vβ2 (51) Vβ18 (32)	2 BP 2 2-28 2 90-170	Vβ2 (60) Vβ18 (14) Vβ14 (12)
Second LP (1 mon later) (CSF 8,085 cells/ml)	Line: 3 BP 2 45-89 5 90-170	Vβ6 (45) Vβ12 (18) Vβ14 (16)	1 BP 2 90-170	Vβ6 (60) Vβ2 (24) Vβ18 (13)
Third LP (4 mon after second LP) (CSF 8,321 cells/ml)	Line: 2 BP 1 peptide	Vβ6 (30) Vβ7 (15) Vβ14 (12) Vβ5.1 (11)	4 BP NT peptide	Vβ6 (20) Vβ2 (15) Vβ18 (10)

SI, stimulation index; Ag, antigen; LP, lumbar puncture; NT, not tested.

Table VI. Summary of Vβ8-CDR3 sequences from Lewis rats with EAE.

	Vβ8 use		$D_{96}S_{97}$				S_{97}			
		1/total sequences	1/total Vβ8.2+[a]	Jβ use	EAE sequence[b]	1 of clones[c]	1/total Vβ8.2[-]	Jβ use	EAE sequence	1 of clones
CSF	Vβ8.2	23/24	11/23	7 Jβ2.7	7/7	4,2	18/24	8 Jβ2.7	7/8	4,2
				3 Jβ1.3	3/3	3		4 Jβ1.3	3/4	3
				1 Jβ2.2	0/1	-		1 Jβ2.2	0/1	-
								4 Jβ1.4	0/4	4
								1 Jβ2.4	0/1	-
	Vβ8.5	1/24	-[d]	-			-	-		
	Vβ8.6	0/24	-	-			-	-		
SC	Vβ8.2	22/22	9/22	6 Jβ2.7	5/6	4	18/22	9 Jβ2.7	5/9	4,3
				2 Jβ1.3	0/2	0		7 Jβ1.3	0/7	3
				1 Jβ2.5	0/1	-		1 Jβ2.5	0/1	-
								1 Jβ1.5	0/1	-
	Vβ8.5	0/22	-	-			-	-		
	Vβ8.6	0/22	-	-			-	-		
LN	Vβ8.2	7/16	1/7	1 Jβ2.7	1/1	-	2/7	1 Jβ2.7	1/1	-
								1 Jβ1.5	0/1	-
	Vβ8.5	5/16	-	-			-	-		
	Vβ8.6	2/16	-	-			-	-		
	Vβ8.7	1/16	-	-			-	-		
	Vβ8.Ψ	1/16	-	-			-	-		

[a] Numbers shown are for Vβ8.2+ sequences only
[b] Number of sequences for each Jβ used which match CDR3β of encephalitogenic T cell clones
[c] Number of cDNA clones with identical nucleotide sequences
[d] Not applicable

Claims:

1. Method for diagnosis of an autoimmune disease in a human comprising the step of detecting the presence of a marker T cell receptor V gene bias in a body fluid which encapsulates all or a portion of a target organ wherein said fluid is selected from the group consisting of cerebrospinal fluid, aqueous humor and the anterior chamber of the eye of a patient.

2. The method of claim 1 wherein said autoimmune disease is a neurological disease.

3. The method of claim 2 wherein said neurological disease is multiple sclerosis.

4. The method of claim 1 wherein said marker T cell receptor V gene bias is for a Vβ gene.

5. The method of claim 1 wherein said marker T cell receptor V gene bias is for Vβ5.

6. The method of claim 1 wherein said marker T cell receptor V gene bias is for Vβ6.

7. The method of claim 1 wherein said marker T cell receptor V gene bias is for Vβ18.

8. The method of claim 1 wherein said marker T cell receptor V gene bias is for Vβ1.

9. The method of claim 1 wherein said marker T cell receptor V gene bias is for Vβ2.

10. The method of claim 1 wherein said body fluid is cerebrospinal fluid (CSF).

11. The method of claim 1 wherein said body fluid is aqueous humor.

12. The method of claim 1 wherein said body fluid is taken from the anterior chamber of the eye.

13. The method of claim 2 wherein said neurological disease is optical nerve damage.

14. The method of claim 2 wherein said neurological disease is anterior chamber inflammation.

15. Method for diagnosis of a neurological disease comprising detecting the presence of a biased motif common to T cell receptors specific for a pathogenic antigen in a non-target tissue or organ.

16. The method of claim 15 wherein said neurological disease is multiple sclerosis.

17. The method of claim 15 wherein said motif is all or part of a marker V gene CDR3 motif.

18. The method of claim 15 wherein said pathogenic antigen is myelin basic protein.

19. The method of claim 15 wherein said biased motif includes an Asp(96)-Ser(97) peptide sequence.

20. The method of claim 15 wherein said neurological disease occurs in a human.

21. Method for diagnosis of an immune-related disease that targets the central nervous system comprising the steps of:

a) providing a sample of cerebrospinal fluid;

b) analyzing the Vβ gene repertoire of said fluid; and

c) determining the presence of a Vβ gene bias.

22. The method of claim 21 further comprising the step of performing one or more marker T cell proliferation assays using one or more compounds selected from the group consisting of Concanavalin A (ConA), human basic protein, human basic protein peptides, guinea pig basic protein (Gp-Bp), guinea pig basic protein peptides (Gp-Bp peptides), PPD, PHA, anti-CD3, interleukin-2 (IL-2) and interleukin-4 (IL-4).

23. The method of claim 21 further comprising the step of performing one or more marker T cell proliferation assays using interleukin-2 (IL-2) together with interleukin-4 (IL-4).

24. The method of claim 21 wherein the step of analyzing the Vβ gene repertoire is performed by polymerase chain reaction amplification and wherein the step of determining a V gene bias is performed using a means for quantifying said V gene bias.

25. The method of claim 21 wherein said cerebrospinal fluid sample is provided from a human.

26. The method of claim 21 wherein said Vβ gene bias is determined when more than about 20% of the expanded T cells demonstrate the Vβ5.2 marker gene peptide sequence.

27. The method of claim 21 wherein said Vβ gene bias is determined when more than about 20% of the expanded T cells demonstrate a Vβ6.1 marker gene peptide sequence.

28. The method of claim 21 wherein said Vβ gene bias is determined when more than about 20% of the expanded T

cells demonstrate a peptide sequence specific for a pathogenic antigen.

29. The method of claim 21 wherein said Vβ gene bias is determined when more than about 20% of the expanded T cells demonstrate a CDR3 motif specific for a myelin basic protein epitope.

30. The method of claim 21 wherein said Vβ gene bias is determined when more than about 20% of the expanded T cells demonstrate a CDR3 peptide sequence specific for a pathogenic antigen.

31. Method for selection of one or more therapeutic Vβ peptide sequences for treatment of a disease or condition, comprising the steps of:

a) identifying the presence of a Vβ gene bias in a body fluid which is not the target tissue or organ of said disease or condition; and

b) selecting an immunogenic peptide corresponding to said Vβ gene bias.

32. The method of claim 31 wherein said immunogenic peptide is an amino acid sequence of the marker TCR.

33. The method of claim 31 wherein said immunogenic peptide is an amino acid sequence of the marker TCR including all or a portion of a CDR2 region.

34. Method for treating Multiple Sclerosis (MS) in a patient comprising the steps of: identifying the CDR2 region of a V gene peptide on a T lymphocyte in the cerebrospinal fluid of said patient, and administering a peptide corresponding to the CDR2 region of a V gene peptide which occurs on the surface of a T lymphocyte in said cerebrospinal fluid.

FIG. 1a.

A. CSF

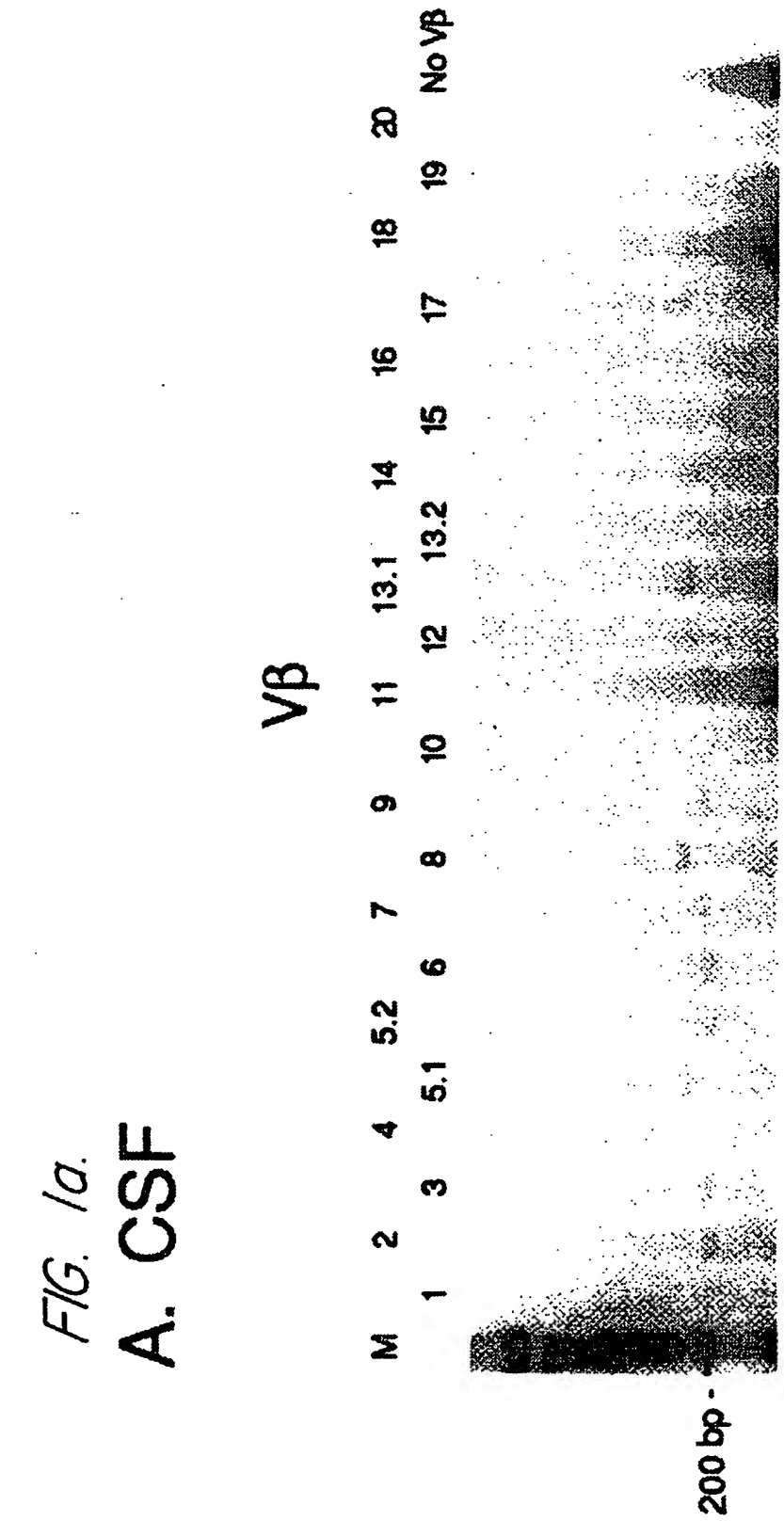

Vβ
M
1
2
3
4
5.1
5.2
6
7
8
9
10
11
12
13.1
13.2
14
15
16
17
18
19
20
No Vβ
200 bp

FIG. 1b.

B. BLD

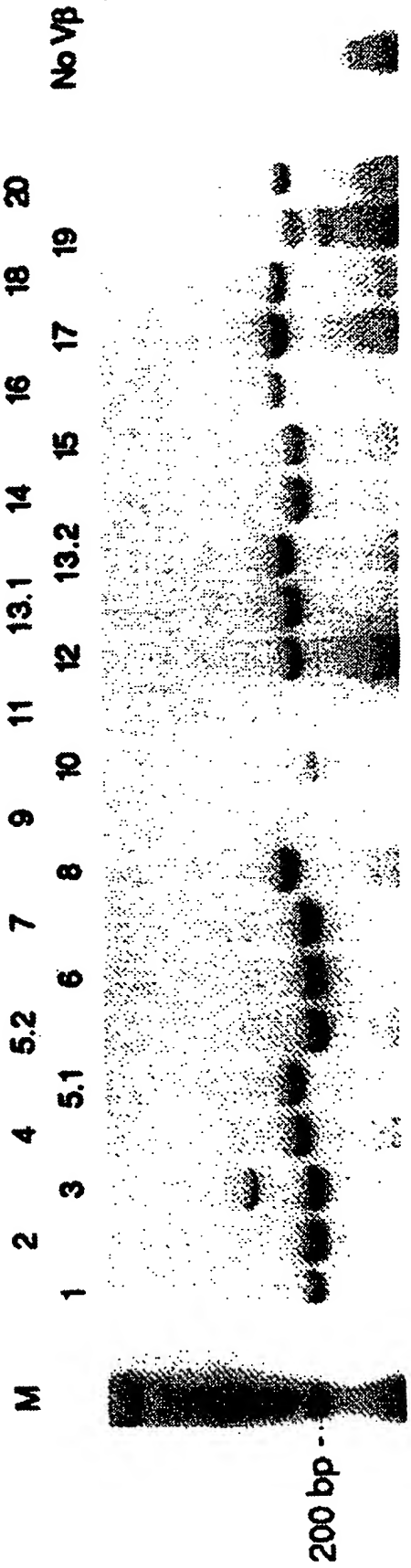

Vβ
M
1
2
3
4
5.1
5.2
6
7
8
9
10
11
12
13.1
13.2
14
15
16
17
18
19
20
No Vβ
200 bp

FIG. 1c.

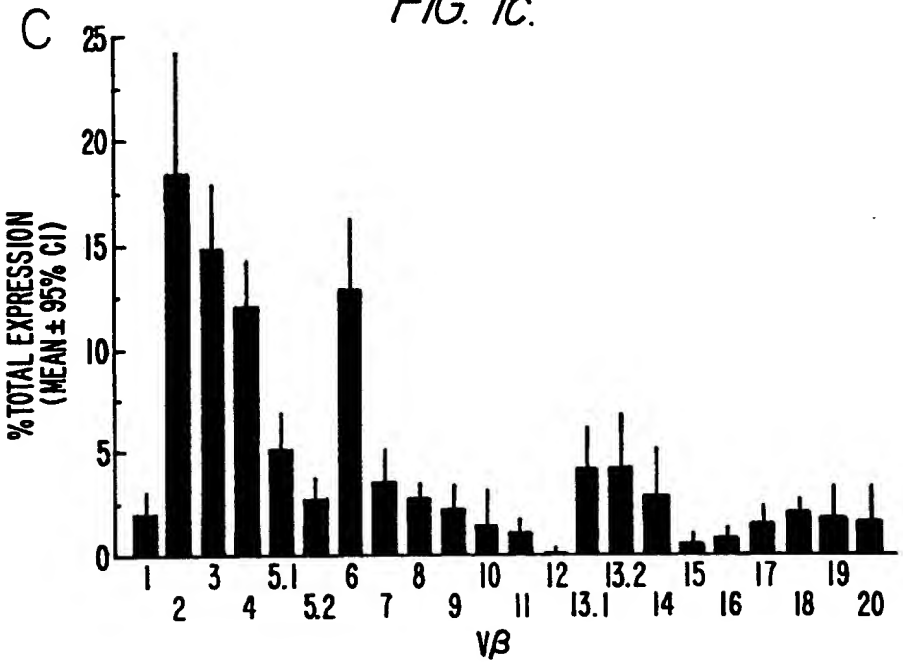


FIG. 2a.

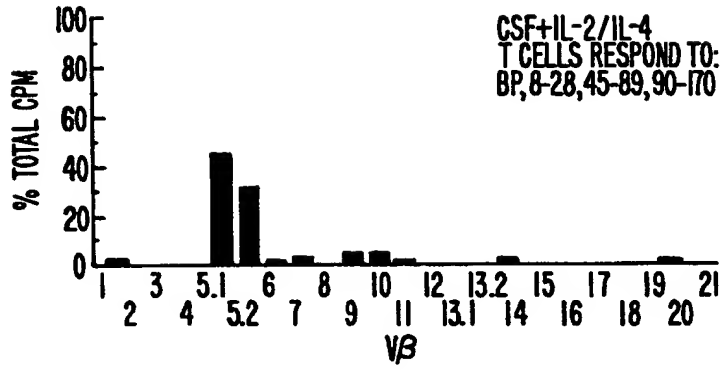
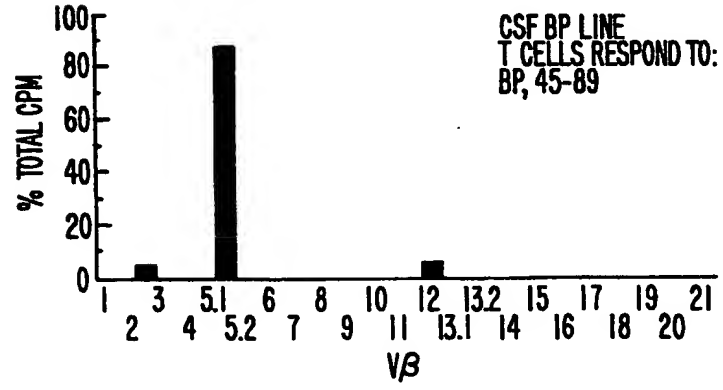
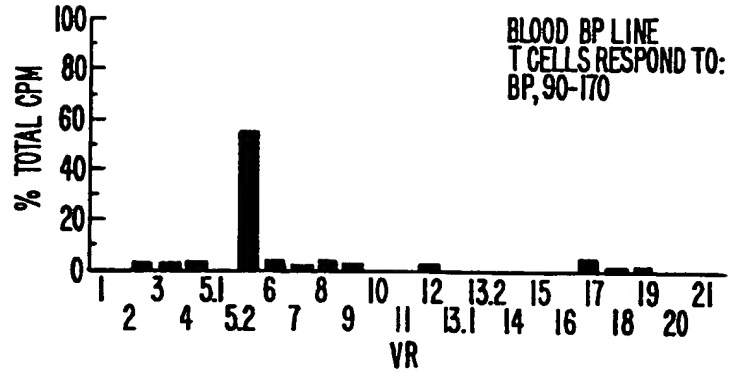


FIG. 2b.



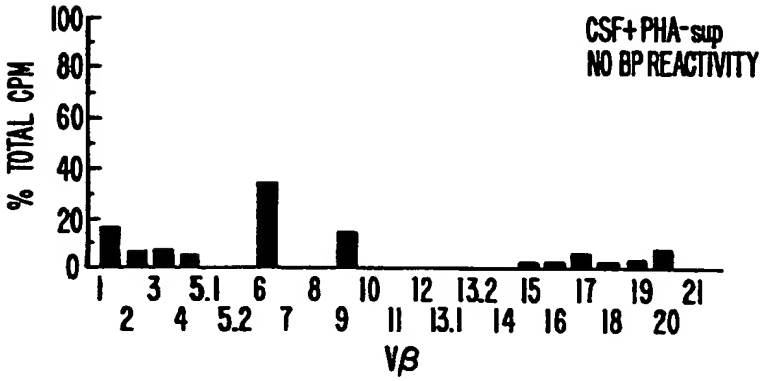
C

FIG. 2c.



D

FIG. 2d.



E

FIG. 2e.

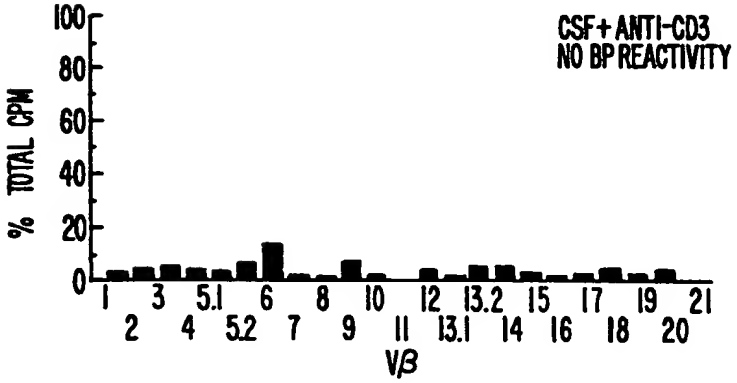


FIG. 3a.

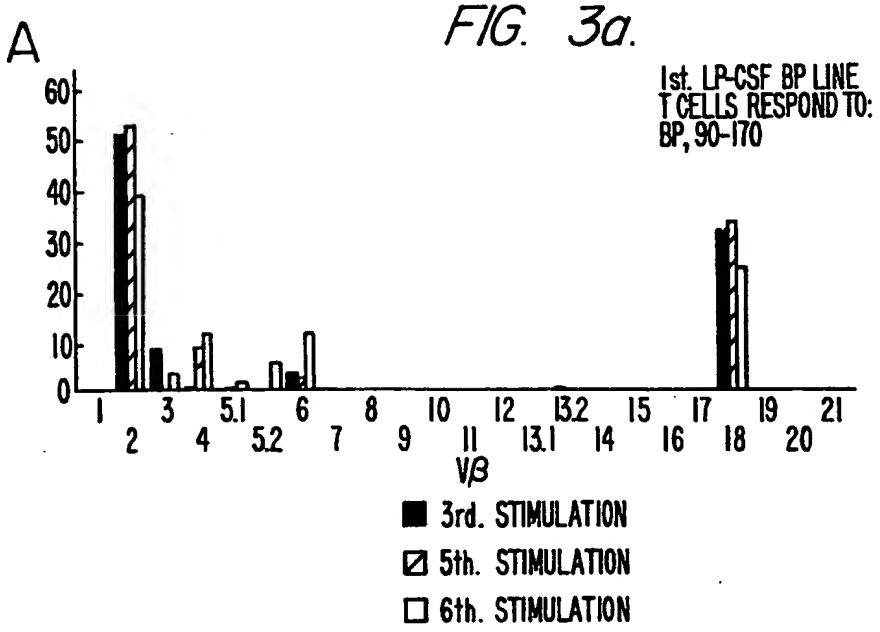


FIG. 3b.

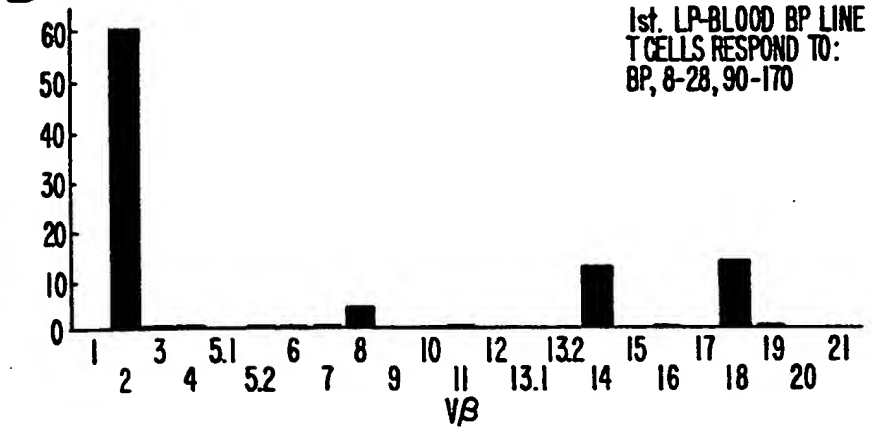
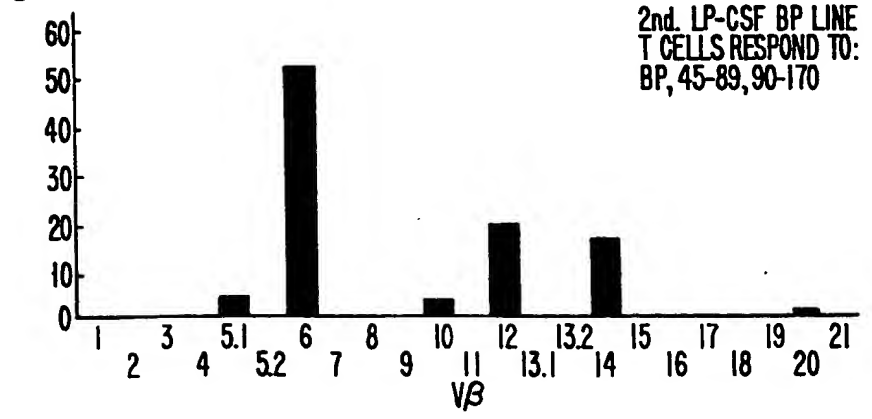
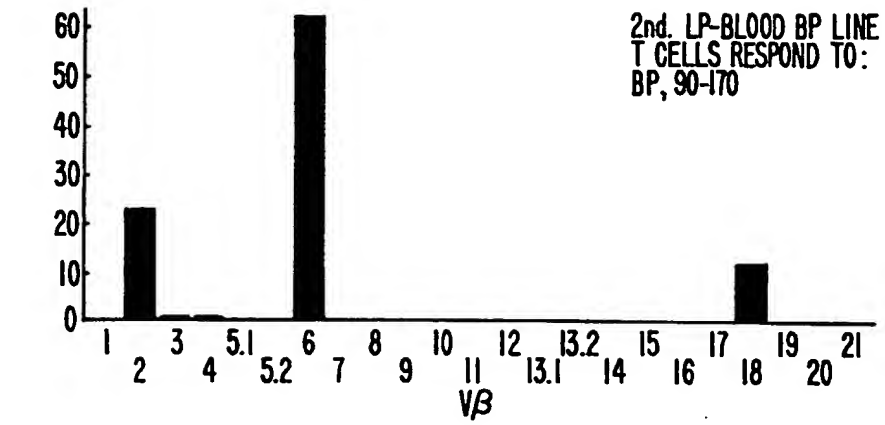


FIG. 3c.



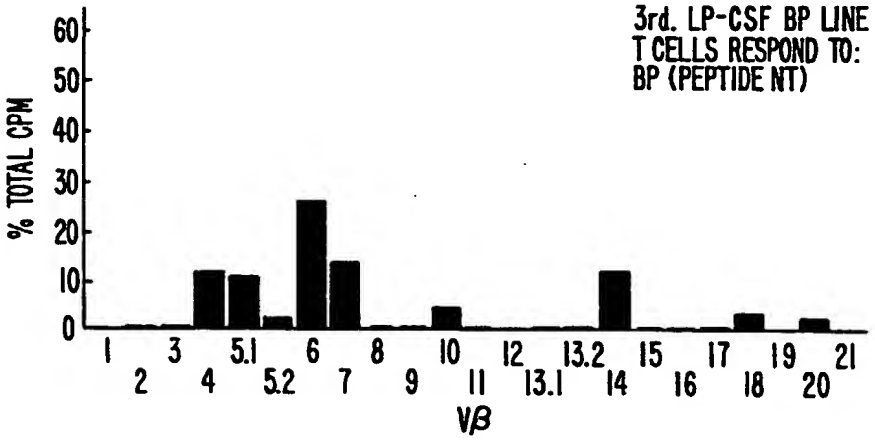
D

FIG. 3d.



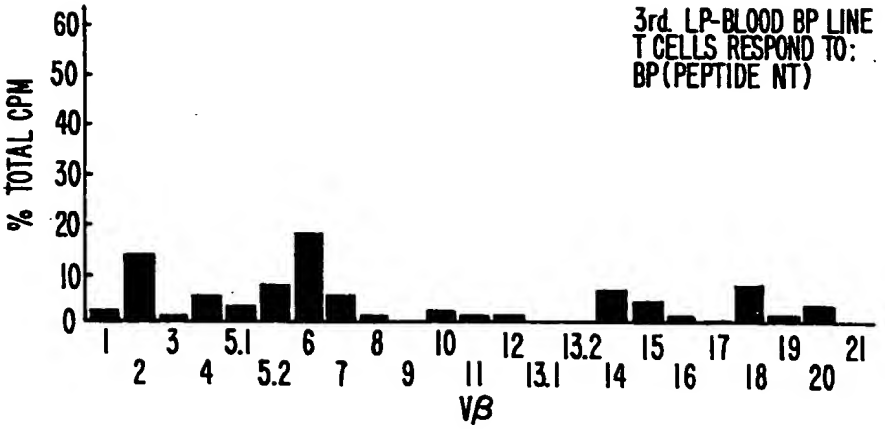
E

FIG. 3e.



F

FIG. 3f.



	N-Dβ-N (96)	… (108)	Jβ	EAE	CDR3 LENGTH	# OF N SEQ	# OF CLONES	
A.								
CSF A2,A3,B15,B16[a]	D S GAC AG	S Y E Q Y F C TCC TAT GAG CAG TAT TTC	2.7	✓	7	0	4	(see SC)
CSF A12	D S GAC TC	S Y E Q Y F C TCC TAC GAG CAG TAT TTC	2.7	✓	7	1	1	
CSF A14,B9[b]	D S S GAT TCC AG	Y E Q Y F C TAT GAG CAG TAT TTC	2.7	✓	7	5	2	
CSF A1,B13,B14[c]	D GA	S G N V L Y F T TCC GGA AAT GTG CTC TAT TTT	1.3	✓	7	0	3	
CSF A10	D S T R A GAC TCC ACC CGG GCA	L Y F CTA TAC TTT	2.2		7	11	1	
B.								
CSF B12[d]	G G GGT GGA	S G N V L Y F TCT GGA AAT GTG CTC TAT TTT	1.3		7	3	1	(see SC)
CSF A6	G G GGC GGT	S Q N T L F F AGT CAA AAC ACC TTG TTC TTC	2.4		7	4	1	
CSF B1,B2,B5,A11[e]	L C CT	S N E R L F F T TCC AAT GAA AGA TTG TTT TTC	1.4		7	3	4	
CSF A8	S S ACT AG	S Y E Q Y F C TCC TAT GAG CAG TAT TTC	2.7		7	2	1	
C.								
CSF B6[f]	M G Q G TG GGA CAG GGA	Y E Q H F TAT GAG CAG CAT TTC	2.7		7	2	1	(see SC)
CSF B11	E E T N GAG GAG ACA AAT G	A N T G Q L Y F CA AAC ACC GGG CAG CTA TAC TTT	2.2		11	9	1	
CSF A9	N Q D R S S AC CAG GAC CGA TCT AG	T D K I Y F T ACA GAC AAG ATA TAT TTT	2.3		10	12	1	
CSF A16	R R A G D AGA CGG GCC GGG GAT	T L F F ACC TTG TTC TTC	2.4		8	11	1	
CSF A15	P G D L CCG GGA GAC CT	Y E Q Y F C TAT GAG CAG TAT TTC	2.7		8	7	1	
CSF A4[*]	E R G G G GAG AGG GGG GGG GGA	E T Q Y F GAG ACC CAG TAC TTT	2.5		9	9	1	

FIG. 4a.

FIG. 4b.

FIG. 4c.

	N-Dβ-N 96	… 108	Jβ	EAE	CDR3 LENGTH	# OF N SEQ	# OF CLONES	
A.								
SC C7,C15,D4,D9[a]	D S GAC AG	S Y E Q Y F C TCC TAT GAG CAG TAT TTC	2.7	✓	7	0	4	(see CSF)
SC C4	D S GAT AG	S Y E Q Y F C TCC TAT GAG CAG TAT TTC	2.7	✓	7	3	1	
SC D2	D S A L GAC TCG GCG CT	E Q Y F T GAG CAG TAT TTC	2.7		7	7	1	
SC C16	D S G R GAC TCA GGG CGA	E T Q Y F GAG ACC CAG TAC TTT	2.5		8	7	1	
SC D13	T D S CT GAC AG	S G N V L Y F T TCT GGA AAT GTG CTC TAT TTT	1.3		8	2	1	
SC C11	T D S CC GAC AG	S G N V L Y F T TCT GGA AAT GTG CTC TAT TTT	1.3		8	2	1	
B.								
SC C1,C12,D15[d]	G G GGT GGA	S G N V L Y F TCT GGA AAT GTG CTC TAT TTT	1.3		7	3	3	(see CSF)
SC C14[g]	G S GGG TC	N Q A Q Y F C AAC CAG GCC CAG TAT TTT	1.5		7	2	1	(see LN)
SC C6,C8,D14[h]	A S GCT AG	S Y E Q Y F C TCC TAT GAG CAG TAT TTC	2.7		7	3	3	
SC C13	S C AG	S G N V L Y F T TCT GGA AAT GTG CTC TAT TTT	1.3		7	0	1	
SC C5	T G P CC GGG CC	S G N V L Y F T TCT GGA AAT GTG CTC TAT TTT	1.3		7	4	1	
C.								
SC D12[f]	M G Q G TG GGA CAG GGA	Y E Q H F TAT GAG CAG CAT TTC	2.7		7	2	1	(see CSF)
SC C3	L S N C TTG TCA AAC	T G Q L Y F ACC GGG CAG CTA TAT TTT	2.2		7	10	1	
SC D7	R T G D CGA ACA GGG G	D Y T F AT GAC TAC ACC TTC	1.2		7	3	1	
SC C9	G T G C GGG ACA GGG	T D Q I Y F ACA GAC CAG ATA TAT TTT	2.3		8	1	1	

FIG. 5a.

FIG. 5b.

FIG. 5c.

	N-Dβ-N 96	108	Jβ	EAE	CDR3 LENGTH	# OF N SEQ	# OF CLONES	
A.								
LN E2	D S GAC TCA	S Y E Q Y F TCC TAT GAG CAG TAT TTC	2.7	✓	7	2	1	FIG. 6a.
B.								
LN F6[9]	G S GGG TC	N Q A Q Y F C AAC CAG GCC CAG TAT TTT	1.5		7	2	1 (see SC)	FIG. 6b.
C.								
LN F5	E R G D GAG AGG GGG GAC	E R L F F GAA AGA TTG TTT TTC	1.4		8	7	1	
LN E15	R G D R A GGA GAC AGA	N E R L F F AAT GAA AGA TTG TTT TTC	1.4		8	5	1	
LN F4	E F P T G GAG TTC CCT ACA GG	Q A Q Y F C CAG GCC CAG TAT TTT	1.5		9	9	1	
LN F11	E A A R T GAG GCC GGC AGG AC	S P L Y F T TCG CCC CTC TAC TTT	1.6		9	10	1	
LN F2	E G GAG GG	T D K I Y F T ACA GAC AAG ATA TAT TTT	2.3		7	1	1	
LN E13*	D S GAC AG	T D K I Y L T ACA GAC AAG ATA TAT CTT	2.3		6	0	1	
LN E11*	V G G F GTT GGA GGG TT	D Y T F T GAC TAC ACC TTC	1.2		7	7	1	FIG. 6c.
LN F9	G T G G G GGG ACA GGG GGA GG	D Y T F T GAC TAC ACC TTC	1.2		8	3	1	
LN F14*	R S G T G TCC GGG ACA T	S N T E V F F CA AAC ACA GAA GTT TTC TTT	1.1		9	5	1	
LN E8*	E G G GAG GGC GGA	S Y A E Q F F TCC TAT GCT GAA CAG TTC TTC	2.1		9	5	1	
LN F7#	A L G G C GCA CTG GGG GGA	K E T Q Y F AA GAG ACC CAG TAC TTT	2.5		9	5	1	
LN F16#	P L G G A CCT TTA GGG GGG GCG	V F F GTT TTC TTT	1.1		7	9	1	
LN F3?	E G Q G GAG GGA CAG GG	Y D Y T F C TAT GAC TAC ACC TNC	1.2		8	2	1	
LN F12ψ	D L G E G GAT TTG GGG GAG GGA G	T Q Y F ACC CAG TAC TTT	2.5		8	10	1	

INTERNATIONAL SEARCH REPORT

Inter al Application No
PCT/US 95/08086

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 G01N33/50 G01N33/68 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF NEUROSCIENCE RESEARCH, vol. 37, no. 2, February 1994 pages 169-81, CHOU, Y. ET AL 'T cell receptor v-beta gene usuage in the recognition of myelin basic protein by cerebrospinal fluid- and blood-derived T cells from patients with multiple sclerosis'	1-10, 21-25
Y	see the whole document	15-20, 31-33
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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
25 September 1995	17. 10. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Osborne, H

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Y	J. IMMUNOLOGY, vol. 152, no. 5, 1 March 1994 pages 2520-9, CHOU Y ET AL 'Immunity to TCR peptides in multiple sclerosis' see the whole document	1-4, 15-20, 31-33
Y	J. IMMUNOLOGY, vol. 152, 1 March 1994 pages 2510-19, BOURDETTE D ET AL 'Immunity to TCR peptides in multiple sclerosis' see the whole document	1-4, 15-20, 31-33
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Y	REGULATORY PEPTIDES, vol. 51, May 1994 pages 77-90, OFFNER, H ET AL 'immunity to cell receptor peptides: theory and applications' see the whole document	31-33
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INTERNATIONAL SEARCH REPORT

Inter al Application No
PCT/US 95/08086

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter...al Application No
PCT/US 95/08086

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